

General Disclaimer

One or more of the Following Statements may affect this Document

- This document has been reproduced from the best copy furnished by the organizational source. It is being released in the interest of making available as much information as possible.
- This document may contain data, which exceeds the sheet parameters. It was furnished in this condition by the organizational source and is the best copy available.
- This document may contain tone-on-tone or color graphs, charts and/or pictures, which have been reproduced in black and white.
- This document is paginated as submitted by the original source.
- Portions of this document are not fully legible due to the historical nature of some of the material. However, it is the best reproduction available from the original submission.

CR-171786
C.1
(NASA-CR-171786) A STUDY OF STRESS-FREE
LIVING BONE AND ITS APPLICATION TO SPACE
FLIGHT Final Report (Baylor Coll. of
Medicine) 47 p HC A03/MF A01

N84-26275

CSCL 06P

Unclas

G3/52 19379

A STUDY OF STRESS-FREE LIVING BONE AND
ITS APPLICATION TO SPACE FLIGHT

Contract NAS9-16442 (Exhibit A)

FINAL REPORT - DECEMBER 1983



A STUDY OF STRESS-FREE LIVING BONE AND ITS
APPLICATION TO SPACE FLIGHT

Contract NAS9-16442 (Exhibit A)

FINAL REPORT - DECEMBER 1983

DEPARTMENT OF MEDICINE

AND

DIVISION OF PLASTIC SURGERY

BAYLOR COLLEGE OF MEDICINE

INVESTIGATORS:

Adrian LeBlanc, Ph.D

Melvin Spira, M.D.

INTRODUCTION

Observations of animals and human subjects in weightless space flight have documented altered bone metabolism. Interpreting these results and the development of effective laboratory models is difficult because many of the mechanisms involved are not understood.

Bone metabolism is affected by a number of local and systemic factors. Examples of local factors are mechanical forces including bending, tension and compression, pH, electrical charge and blood supply. Systemic factors include Vitamins D and C, hormones, cortisol, PTH, estrogen, calcitonin thyroxine, Ca^{++} supply resulting from changes in absorption or excretion, dietary factors such as low Ca^{++} intake, high phosphorus or high protein intake, and nonspecific changes such as those related to stress.

The calcification and growth of transplanted bone is independent of local muscle, nervous and mechanical forces, and therefore transplanted bone would provide data on the role of local vs. systematic factors.

The goals of this work were to investigate bone metabolism in living transplanted bone, devoid of stress, as a possible tool for the investigation of countermeasures against disuse bone loss.

BACKGROUND

Three of the nine Skylab crewmembers lost statistically significant bone mineral from the os calcis¹. No loss was seen in the radius and ulna which is assumed to be related to the low percentage of trabecular bone at these sites. A preliminary five-year followup study of the nine flight crewmembers demonstrated a statistically significant loss of bone mineral during the interval in the flight crew while the controls did not². The three crew-

members that demonstrated a statistically significant loss of bone mineral during flight also had above average loss of calcium in the balance study. Classically, disuse osteopenia has been thought to be due to mechanical factors such as the absence of pressure transmitted to bone or the absence of tensile and shearing forces applied to bone by muscle³. Intermittent pressure on the os calcis during programmed bicycle and treadmill exercise in Skylab might have affected the calcium loss. However, bedrest studies have shown generally that intermittent pressure on the feet does not prevent calcium loss. In bedrest studies even 3 hours of quiet standing per day, or a similar period of recumbent bicycle ergometry exercise fails to reverse the negative calcium balance^{4,5}. Vogel, et al, who analyzed and performed the photon absorption densitometry of the os calcis, radius and ulna, noted that calcium loss from these narrowly defined bone areas was of similar degree to that found in bedrest of equal length⁶. Since the negative calcium balance of Skylab crewmembers tended to be greater than that recorded in bedrested subjects, we can postulate that if the exercise had a sparing effect on bones measured by densitometry, then the bulk of the calcium loss noted in the balance study came from other areas of the skeleton.

The three manned Skylab missions did produce evidence of a relationship between the length of the period of weightlessness and bone demineralization. There was no statistically significant loss observed in the three crewmembers of Skylab 2, in two crewmembers of Skylab 3, and in one crewmember of Skylab 4¹. On the other hand, the amount of daily exercise performed increased from Skylab 2 to 4 such that there was less loss of leg and arm strength and leg volume after Skylab 4 than after Skylab 2. Therefore, the increased exercise improved muscle condition, but apparently did not prevent a negative calcium balance. Comparing the calcium balance data for these three Skylab space missions with the percent loss in the os calcis, there appears to be a

relationship between the amount of Ca lost by the balance method and the mineral loss in the os calcis.

Within one day following insertion of the Skylab crewmembers into the weightlessness of orbital flight, the quantity of calcium appearing in the urine began to increase. Within 10 days the positive calcium balances which prevailed preflight were abolished and the body as a whole began to lose calcium⁸. The loss was slow at first, amounting to about 50mg per day at 10 days, with a gradual increase to almost 300mg per day by the 84th day of flight. Examination of the data reveals that at the end of 84 days in flight, the average Skylab crewmember had lost approximately 25g of calcium. From this it is evident that approximately 2.5% of the pool was lost during the mission.

Hypercalciuria may have been produced by the relatively high levels of protein (114g per day) consumed by Skylab astronauts⁹. However, this effect could not be a primary factor since the balance preflight was positive when ingested protein was higher than during the missions. In addition, the elevated levels of phosphorus in the diet (1700 - 1800mg per day) are known to markedly reduce urinary calcium with little or no change in apparent absorption of calcium when a high protein diet is consumed¹⁰. Within thirty days following the onset of weightlessness, the quantity of calcium appearing in the urine leveled out at approximately 100 percent above preflight levels. This level of excretion was maintained for the remainder of the flight. During the post-flight period the quantity of calcium excreted in the urine fell rapidly to levels lower than preflight. The calcium content of the stool exhibited a quite different pattern. It fell initially to levels below preflight and thereafter rose in an apparently linear fashion until the end of the flight, after which it tended to return toward preflight levels.

In the Skylab flights an increase in bone resorption or a decrease in bone formation might have led to the observed increase in serum calcium and phosphorus concentrations¹¹. These elevations were followed by increased urinary concentration of these elements (either by increasing the filtered load or by suppressing parathyroid hormone secretion).

The mechanism for the fecal calcium increase is less well defined. Calcium absorption is controlled to a large extent by the hormonal metabolite of vitamin D₃, 1,25-DHCC. The 1,25DHCC, besides stimulating renal reabsorption, stimulates calcium absorption¹². This metabolite was not measured, but its 25 hydroxylated precursor was measured. It was slightly decreased by post-flight in the Skylab 4 crewmembers, but unchanged in the other six Skylab members¹¹. The C terminal metabolite of parathyroid hormone initially showed slight, but not statistically significant, increase in flight concurrent with statistically significant increase in serum calcium¹¹. The free cortisol changes do not seem great enough to have caused a significant loss of bone substance since the values are considerably below levels found in Cushing's disease, and the duration effect was short for the calcium balance change noted in Apollo and Gemini crews. The same is true of thyroid hormone changes. Aldosterone excretion increased two to three times; serum sodium decreased about 4meq/l, and the plasma volume decreased about 10%. These changes would affect resorption of calcium by the kidney. Although the data suggest the development of progressive malabsorption of calcium, the possibility of increasingly large amounts of calcium secreted into the gastrointestinal tract cannot be discounted.

The fundamental mechanism by which calcium loss occurs is presumed to be primarily increased bone resorption rather than decreased bone formation. In view of this generally held belief, it is surprising that during the 27 day Cosmos 602 flight, investigators noted an increase in lacunar pore diameter

over ground based controls and a decreased rate of periosteal bone formation of approximately 40% less than controls. An arrest line was found at both the endosteum and periosteum of the flight animals, suggesting that a complete cessation of bone growth occurred during flight¹³. Microscopic examination of the femur, tibia, and humerus of Wistar rats following a 19.5 day space flight aboard Cosmos 782 showed decreased metaphyseal bone which was actually combined with a decrease in spongy mass in the vicinity of the epiphyseal cartilagenous plate, suggesting an inhibition of bone growth during flight¹⁴. These results are viewed somewhat equivocally because of the observation that centrifugation of rats at 1-g on the 18 day flight of Cosmos 936 failed to correct the retardation of bone formation¹⁵.

In these Cosmos flights the well known effects of changes in nutrient intake on bone growth cannot be completely ruled out since the flight rats gained less weight than ground-based controls. It is recognized, for example, that changing from a 0.6% to 0.025% calcium diet can cause a significant decrease in bone mass¹⁶. In addition, caloric reduction can have dramatic effect on the bone growth in young rats. For example, a 50% caloric restriction, even with normal calcium intake, can cause complete cessation of skeletal growth¹⁷.

During space flight, in addition to the absence of gravity, an altered electromagnetic environment is encountered. During Earth orbital flight, the motion of the spacecraft through the geomagnetic field induces an extremely low-frequency electric field. It has been hypothesized that this altered electromagnetic environment has a causative role in bone calcium loss¹⁸. Electric fields have altered circadian rhythms and reaction time in humans, and have depressed the rates of growth and fracture healing in rats^{19, 20, 21, 22}.

There is a vast body of literature that concerns itself with defining the blood supply to bony tissue. In conventional bone grafts, (i.e. bony "transplantation"), during which time cortical bone is separated from its nutrient

supply, most of the osteocytes die and the matrix of the bone serves merely as a scaffold for ingrowing host cells with osteogenic properties²³. Grafts transplanted into soft or bony tissue with intact periosteal and medullary circulation do not undergo osteocytic death and "creeping substitution", rather, they undergo complete revascularization²⁴. Bone thus transferred is independent of surrounding tissue and remains organized and alive retaining its original size and form.

In the laboratories of Baylor College of Medicine, Division of Plastic Surgery, an animal model was developed for transplantation of bone on a nutrient vascular pedicle²⁵. This model, which uses Sprague-Dawley rats, entails resecting the distal half of the femur with placement (i.e. "transplantation") of the resected femur, on its nutrient vascular pedicle, into the groin of the animal. We have shown that this model undergoes no necrosis or creeping substitution, making it ideal for this study²⁶. The very uniqueness of the experimental model makes it ideally suited for a study of bone and bone metabolism under conditions which closely simulate those which may be encountered in long term space flight.

This unique model has a number of advantages for studying a wide variety of factors which could alter bone metabolism. Most animal models used to examine bone disuse necessitate some form of restraint, e.g. casting or suspension, which invariably produces stress with accompanying physiological changes. Using this particular transplanted bone model, stress producing immobilization of the animal is not required.

The transplanted bone is subject to gravitational forces but isolated from neuromuscular influences, and is therefore useful for the study of g-forces on bone, e.g., increased g-forces, bone/gravity orientation. The transplanted bone model could be used to study how mechanical forces affect growth and calcification without disruption of its own blood supply. Later studies with this model

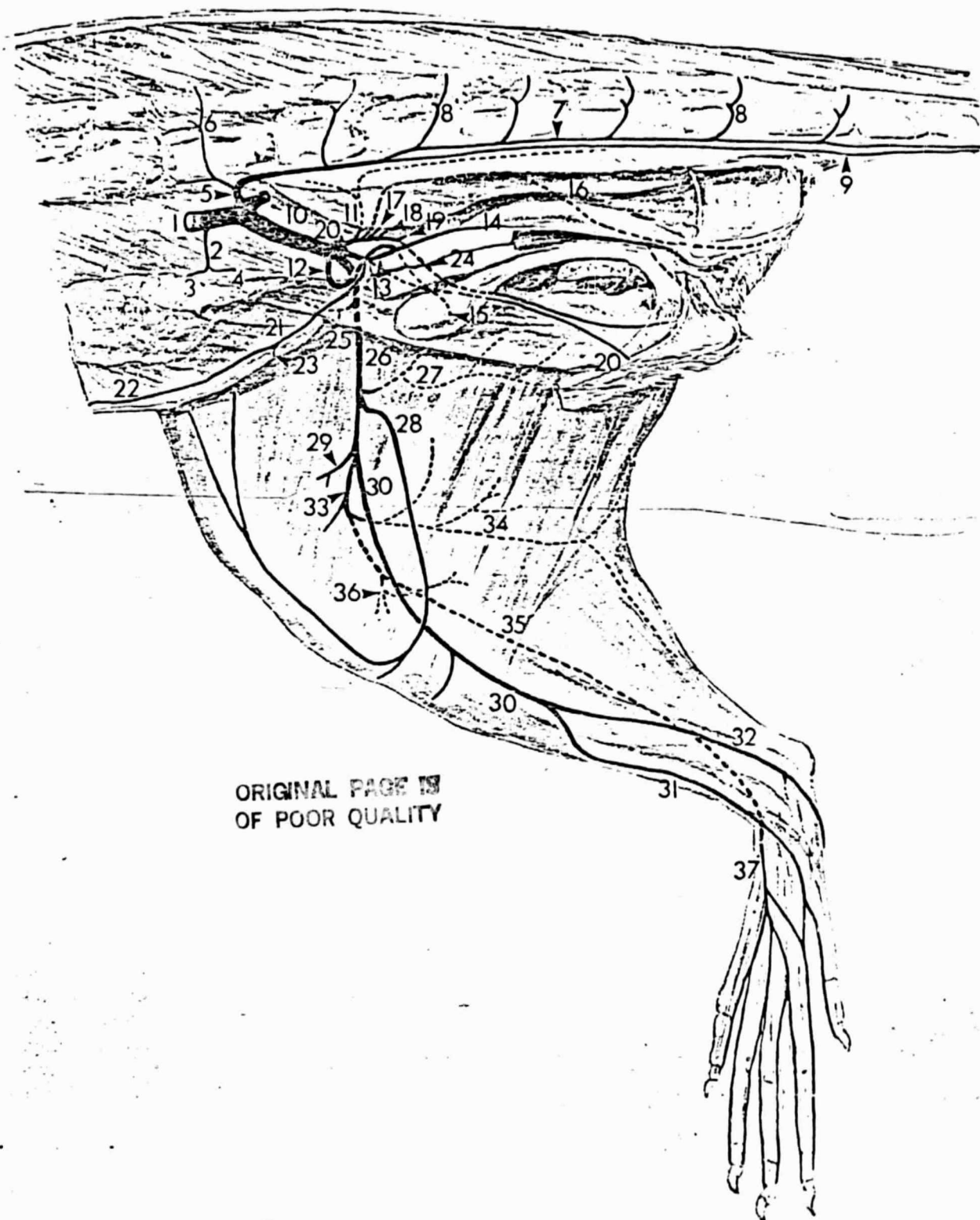
could include the healing of bone fractures, the resistance of bone infection under these unique conditions, the regrafting of the implant back to a long term bone to investigate remobilization effects, and the long term (2 to 3 years) effects on transplanted bone.

METHODS

SURGICAL PROCEDURE:

The Sprague-Dawley rat (250-360g) is used as the animal model for transplantation of bone on a nutrient vascular pedicle. This procedure entails resecting the distal half of the femur with placement of the resected femur, on its nutrient vascular pedicle, into the groin of the animal.

A skin incision is made from the patella of the knee to the pubic bone exposing the femoral vasculature as in a routine groin dissection (Fig.1). The epigastric vessels must be freed in order to prevent vascular tension. Just below the epigastric vessels there is a small white line of fascia extending toward the knee. This is the starting point for dissection of the entire inferior half of the femur on the ventral side of the animal. This line is followed very carefully separating the plane between the muscle adductor longus and the muscle adductor magnus et brevis (Fig.2). Dissection is continued until the femur bone comes into view paying special attention to preserve both lateral and medial superior geniculate and the central intercondylar vessels. The geniculate vessels feeding the muscles above the bone may be cauterized, freeing the bone superiorly. The white line is followed all the way to the patella tendon. The insertion of the cranial and caudal portions of the gracilis muscle are transected just medial to the patella tendon so that they may be retracted back. This then exposes the origin of the medial gastrocnemius which is also resected cauterizing any vessels encountered. The femur bone muscles are detached from the bone, cauterizing any vessels feeding them, being careful not to sacrifice the saphenous nerve. The muscles may be detached inferior to the bone



ORIGINAL PAGE IS
OF POOR QUALITY

Fig. 1 Arteries of the right pelvic limb, medial aspect. Broken lines indicate deeper portions of the vessels; for reasons of clearness the plantar vessels of the foot are not shown. 1, aorta abdominalis; 2, a. mesenterica caud.; 3, a. colica sin.; 4, a. rectalis; 5, a. sacralis mediana; 6, r. sacralis; 7, a. sacralis lat.; 8, its segmental branches; 9, a. caudalis mediana; 10, a. iliaca; 11, a. glutea cran.; 12, a. umbilicalis; 13, a. uterina; 14, a. vaginalis; 15, a. vesicalis cran.; 16, a. pudenda int.; 17, a. circumflexa femoris lat.; 18, a. circumflexa femoris med.; 19, a. obturatoria; 20, tr. pudendoepigastricus; 21, a. epigastrica caud.; 22, its r. dors.; 23, its r. ventr.; 24, a. pudenda ext.; 25, a. circumflexa ilium sup.; 26, a. femoralis; 27, a. profunda femoris; 28, a. epigastrica sup.; 29, a. genus desc.; 30, a. saphena; 31, r. tarsalis med.; 32, branch to plantar vessels; 33, a. poplitea; 34, a. femoris caud.; 35, a. tibialis cran.; 36, a. tibialis caud.; 37, a. dorsalis pedis. (Drawing by B. Ruppel.)

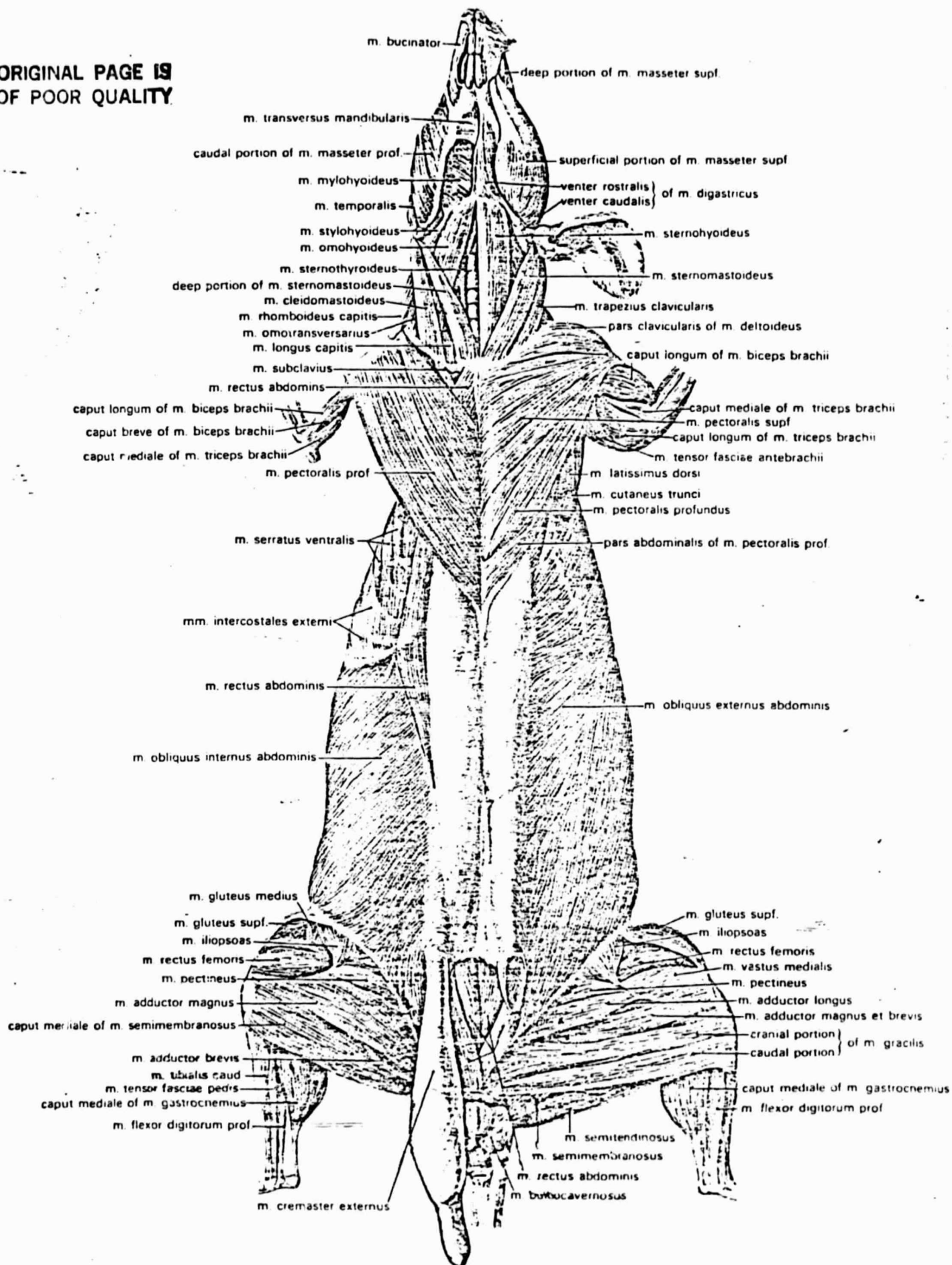


Fig. 2 Superficial (left side) and deep muscles (right side), ventral aspect

sacrificing the saphenous artery and vein. The popliteal vessels will now come into view and are dissected by sacrificing the small muscle covering them being careful to cut close to the knee to avoid any branches from these vessels. Elevating the vastus medialis muscle, a small incision is made into the capsule of the knee opening the joint by following the curve of the condyle. The position of the leg is now changed to a more lateral position to allow clear view of the popliteal vessels behind the knee. The medial ligament is cut slowly so as to preserve joint cartilage. The posterior capsule is then cut very slowly to avoid cutting the popliteal vein. The capsule is then separated from the popliteal vein at the level of the superior border of the tibia with ligation of the vein immediately under the knee joint as close to the tibia as possible. The posterior capsule dissection is completed by placing the microscissors behind the knee and cutting the posterior cruciate ligament. After this is done the joint will open immediately. The patella tendon is then cauterized and transected. This completes the isolation of the inferior portion of the femur.

At this point, the animal's leg is repositioned by crossing it over the other leg so that a posterior dissection may be achieved. A skin incision is made as a continuation of the ventral incision again exposing a white line of fascia between the rectus femoris, pectineus and abductor magnus muscles (Fig.3). This line is carefully dissected, as before, until the lateral superior geniculate vessels are exposed. The head of the femur is completely isolated by sacrificing three tendons; the insertion of the muscle rectus femoris, the insertion of the muscle adductor magnus, the insertion of caput mediale of the semimembranosus muscle, the lateral ligament to the medial gastrocnemius, and the popliteal muscle tendon. Staying high above the femur, the muscle rectus femoris is removed from the femur ligating any small vessels feeding that muscle; continue down the femur avoiding sacrifice of the lateral superior geniculate vessels supplying the head of

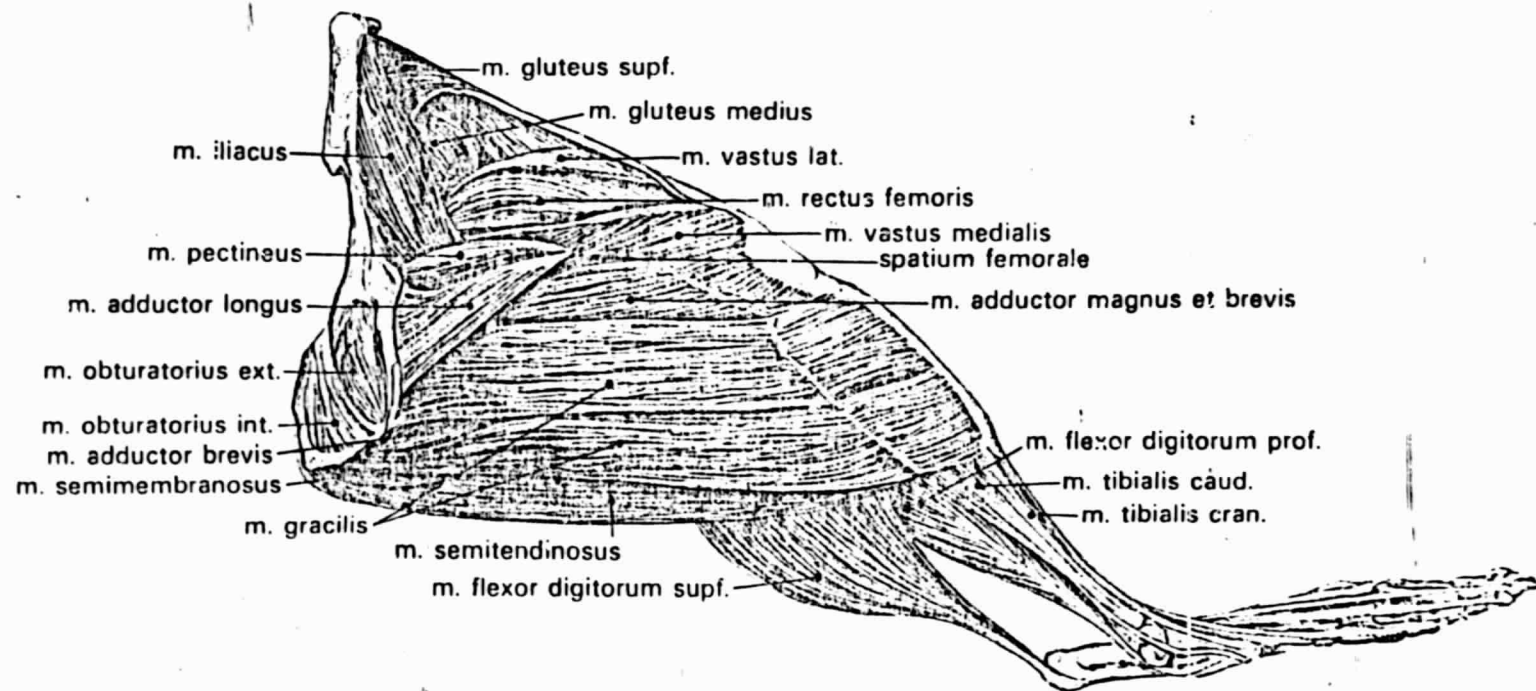


Fig. 3 Muscles of the pelvic limb, medial aspect

the femur. At this point the femur is completely exposed for a length of up to 3cm. The anterior cruciate ligament is then cut at the head of the femur allowing complete mobility of the bone. The head of the femur is held carefully and a 2cm segment is measured with Vernier calipers (Fig.4). The femur is then sectioned at the junction of the medial and inferior third of the shaft with a Micro-air digital saw leaving the bone vascularized only by the femoral vessels connected to the lateral and medial superior geniculate and central joint vessels. The head of the femur is then inspected for bleeding at the cut end so as to indicate patency of the lateral and medial superior geniculate and central joint vessels. The bone is then transplanted to the free groin space (Fig.5). The remaining two ends of bone are connected with a blunt #18 gauge needle to splint the two bones to allow some mobility of the leg by the animal. The skin incision is closed with nylon suture.

BONE DENSITOMETRY:

The technique used is a modification of the method described by Cameron, et.al.²⁷ The technique uses an I-125 sealed source to image the bone using a large field of view gamma camera. The image is stored in computer memory with a 64x64 matrix. A jig is used to hold the I-125 source and bone approximately six and five feet, respectively, above the camera detector with the collimator removed. A lead ring is used to shield the edge of the detector crystal. A lead diaphragm is used to collimate the I-125 x-rays to the area of the bone to reduce scatter. The bone to be imaged is submerged in a water trough to provide constant thickness for all portions of the scan. Since the magnified image is stored in computer memory both total and regional bone mineral is possible. A prescan flood is used to correct for detector nonuniformities and a calibration curve using aluminum disks is obtained for each area. Figure 6 shows such a calibration curve relating

ORIGINAL PAGE IS
OF POOR QUALITY

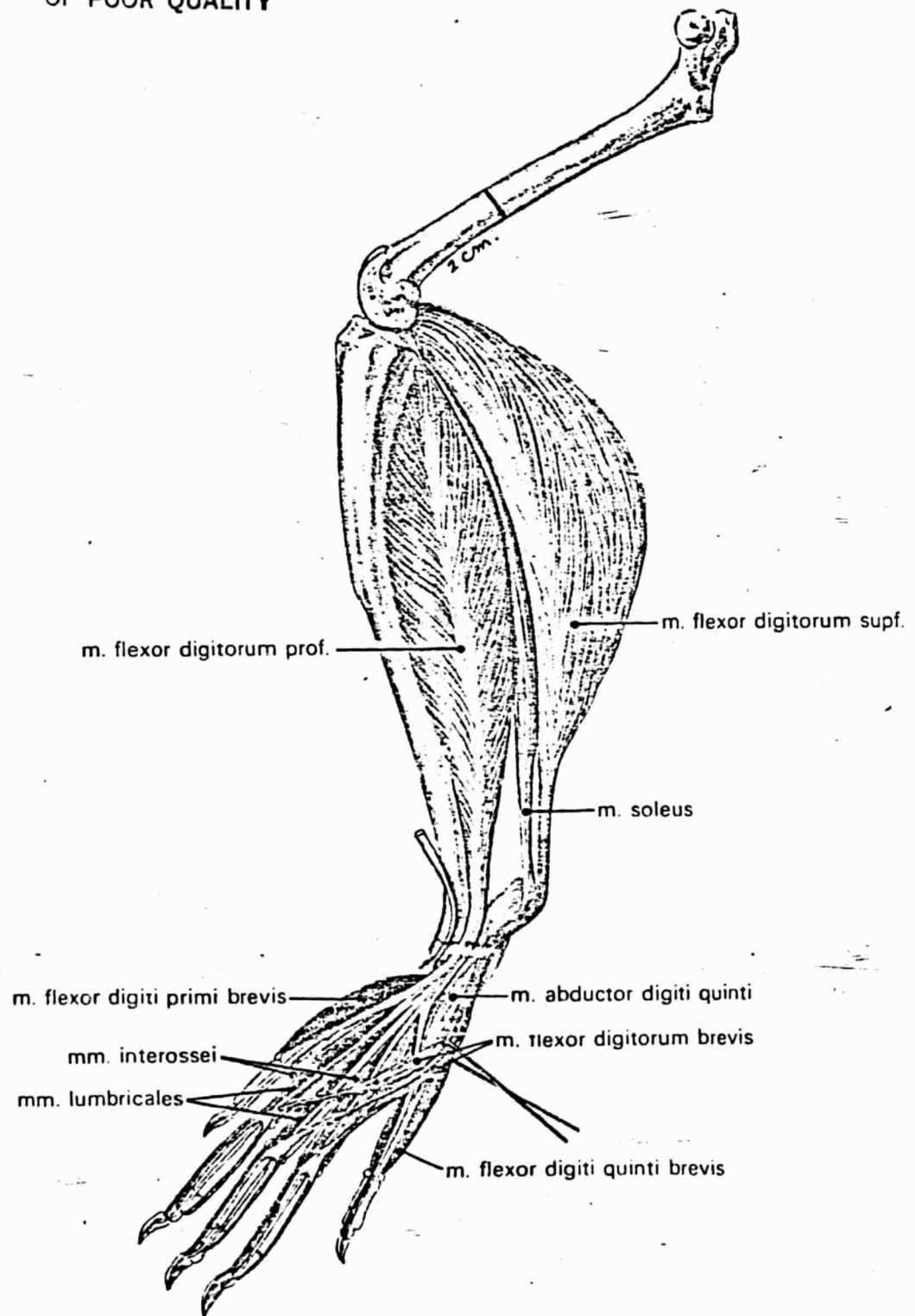


Fig. 4 Muscles of the pelvic limb, medial aspect

ORIGINAL PAGE 19
OF POOR QUALITY

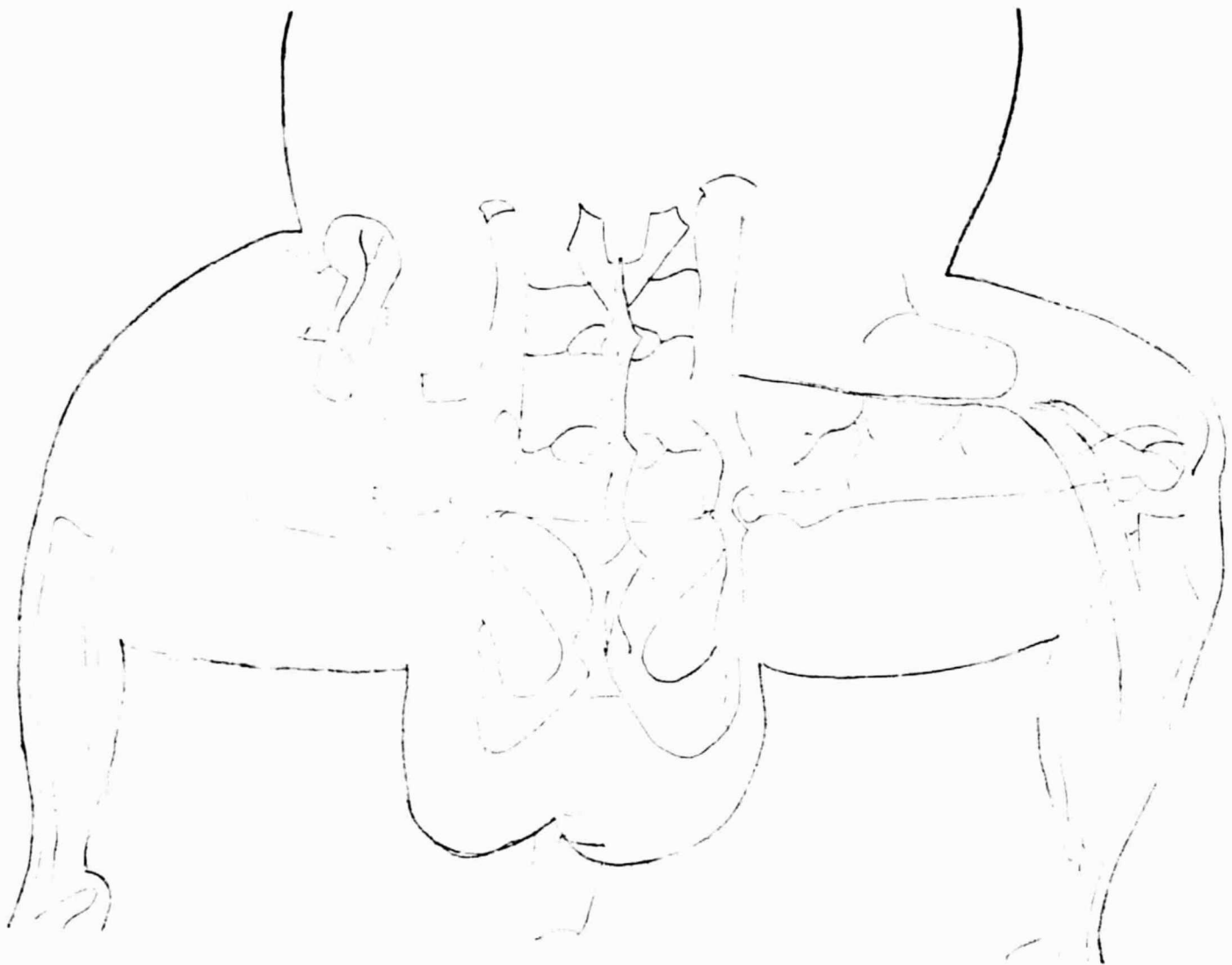


FIGURE 5

The transplanted femur is present in the
right groin of the rat.

(F) - transplanted femur

(P) - vascular pedicle

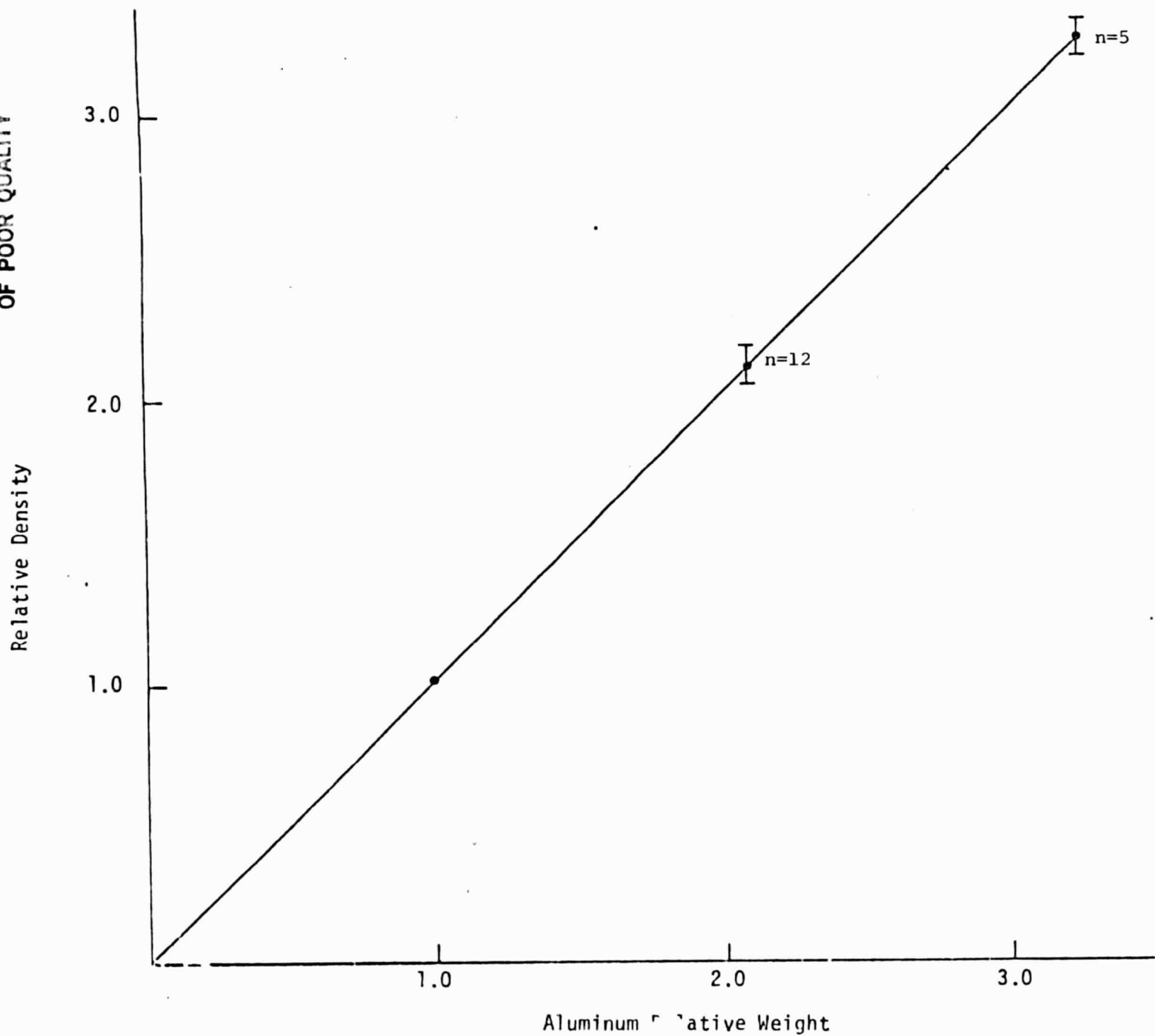


FIGURE 6 Calibration Curve Relating Relative Density vs. Weight of Aluminum

relative density vs relative weight of aluminum. For this study actual grams of bone mineral is not required, therefore arbitrary units can be used to represent density. To obtain a scan (x or y) a sequence of 25 - 30 slices across the bone is obtained, the sum of which gives the total bone density. This technique has been developed and documented and a manuscript describing this technique has been accepted for publication in Physics in Medicine and Biology. A copy of this paper is contained in the appendix.

Tc-99m MDP UPTAKE AND SCAN:

The uptake of Tc-99m phosphates by bone is a function of blood flow and bone remodeling. The quantification of this uptake is useful as an indication of bone viability. In addition, it has clinical importance as a non-invasive technique for investigating changes in bone as it relates to changes in bone remodeling and bone blood flow which are normally intimately related. Four hours prior to sacrifice the rat received a calibrated 5mCi dose of Tc-99m methylene diphosphonate (MDP) via a percutaneous injection into the jugular vein. After sacrifice the control and experimental bones were scanned to determine total and regional activity. Each batch of labeled MDP is checked by chromatography to insure at least 95% binding. The scanning device consists of a lead sheet containing a 1mm slit viewed by a 3" x 3" sodium iodide detector. The detector is connected to a 1024 channel analyzer to record the Tc-99m photopeak counts. The bone scan is achieved by moving the bone sample across the lead slit using a stage micrometer in 1 to 2mm increments.

BONE BLOOD FLOW:

Just prior to sacrifice the rat is anesthetized and a modified 21g heparinized butterfly is inserted percutaneously into the left ventricle. As soon as the left ventricle puncture is achieved, a syringe containing 800,000

Ce-141 or Ru-103 carbonized microspheres is attached to the butterfly and the microspheres flushed through the butterfly into the left ventricle. Two minutes later the animal is sacrificed using an overdose of Nembutal and the experimental and control bones harvested. Two to three days later a scan of the Ce-141 activity is performed in a similar manner as described for Tc-99m MDP. The 2 to 3 day delay is to allow for the decay of the Tc-99m activity. The uptake in the left and right kidneys is also determined to document adequate mixing of blood and microspheres.

RADIOGRAPHIC IMAGING:

High resolution x-ray images are obtained of both control and experimental bones using a Faxitron x-ray machine. Images are obtained at both 30 and 40KEV using 2.5ma for one minute.

EXPERIMENTAL DESIGN

PHASE I:

The purpose of this work was to define the expected physical changes in transplanted bone after various lengths of time following transplantation. A total of 30 female Sprague-Dawley rats received a femur transplant with 17 surviving through the completion of the study. Rats were sacrificed at 4 weeks (n=2), 6 weeks (n=3), 9 weeks (n=3), 18 weeks (n=3), 24 weeks (n=4), and 51 weeks (n=1).

PHASE II:

In Phase I, the pedicled bone demonstrated clear evidence of enhanced bone resorption which was easily distinguishable from simple growth (decrease) changes. This study was conducted as a pilot experiment in order to determine if clomiphene (Clomid) might retard the enhanced resorption of bone. Clomiphene is a synthetic estrogen agonist/antagonist used for many years to induce ovulation in anovulatory women³⁰. In a recent study we

demonstrated that clomiphene had a protective effect against bone loss in ovariectomized aged rats (see appendix). This study was conducted in order to determine if this drug might retard disuse bone resorption in rats with intact ovaries. A total of 11 adult female Sprague-Dawley rats (250 - 300g) received a pedicle graft. Of these, six received weekly 10mg subcutaneous injections of clomiphene (experimental) suspended in 0.2cc of mineral oil while the remainder received no clomiphene. Six of the animals (three experimental and three controls) were sacrificed at six weeks after surgery while the remainder (three experimental and two controls) were sacrificed 10 weeks after surgery.

RESULTS

SURGICAL RESULTS:

Figures 7A, B, and C illustrate the steps in the preparation of a pedicled graft. Figure 8 shows a pedicled graft six weeks after implantation. The nutrient arteries and veins have become noticeably enlarged and the graft is covered with a fibrous sheath. About 15 - 20% of the rats undergoing surgery did not survive the operation for one reason or another. Another complication of the procedure is that the pinned femur/radius connection may become disconnected before adequate healing has taken place. The dislocated pin can cause a localized infection or necrosis requiring sacrifice of the animal. This limitation could be improved using a better method for securing this union.

PHASE I

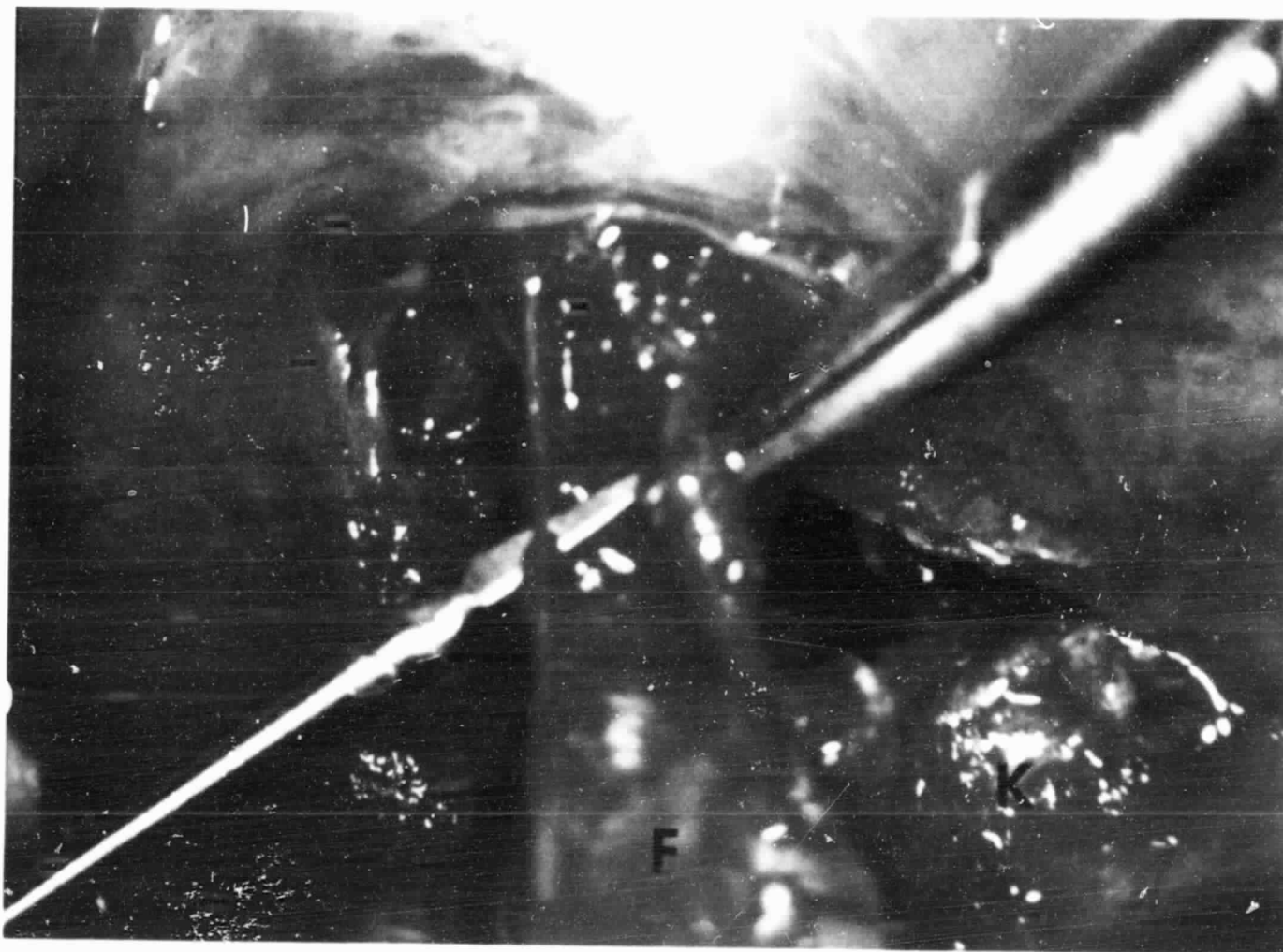
The total bone uptake of Tc-99m MDP and radiolabeled microspheres and the total bone mineral content (BMC) are given in Table 1. These results are expressed as a ratio of pedicle to control bones; the control bone being a portion of the femur from the opposite limb cut to the same length as the pedicle at the time of sacrifice. Since the pedicle implant shortens with



ORIGINAL PAGE
COLOR PHOTOGRAPH

Fig. 1 A Femur (F) is shown with the medial superior geniculate pedicle (M).

Taken under 15 x magnification under the Zeiss OPMI-7PH operating microscope.



ORIGINAL PAGE
COLOR PHOTOGRAPH

ORIGINAL PAGE
COLOR PHOTOGRAPH

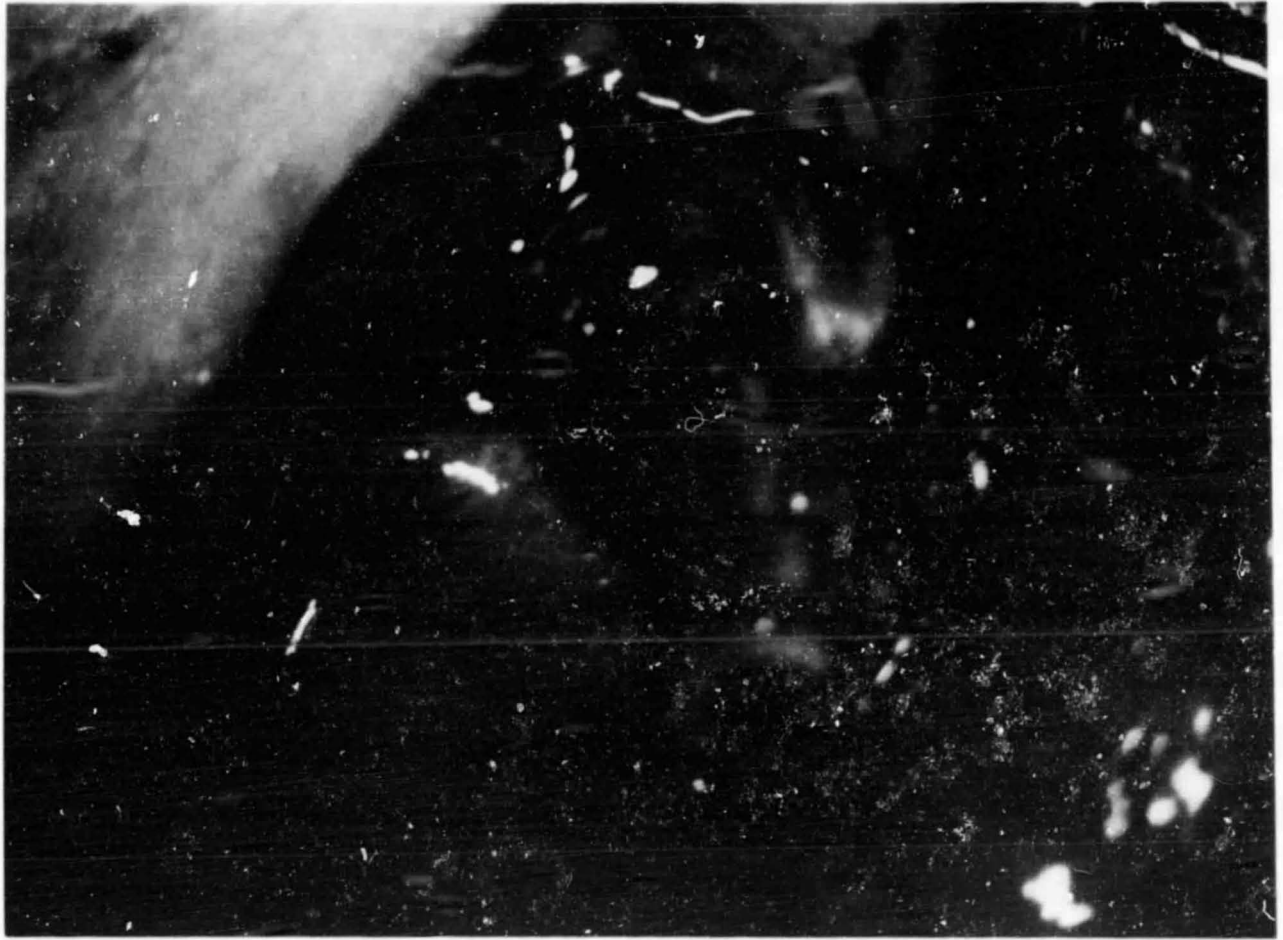


Fig. 1 C Free upper head of femur (F).

Taken under 15 x magnification under the Zeiss OPMI-7PH
operating microscope

TABLE I

Ratio of Pedicle to Control Bone: 4 hr MDP Tc-99m residue, blood flow (microspheres) and bone mineral.

<u>TIME</u>	<u>RAT #</u>	<u>MDP</u>	<u>PEDICLE/CONTROL</u>	
			<u>Microspheres</u>	<u>Bone Mineral</u>
4 weeks	2B	1.45	0.90	1.03
	3B	1.18	0.71	0.99
	Mean	1.32	0.80	1.01
6 weeks	3A	1.55	1.37	0.84
	10A	1.28	1.41	0.78
	21A	1.53	--	0.85
	Mean	1.45	1.39	0.82
9 weeks	4A	1.44	1.71	0.92
	5A	1.36	1.69	0.94
	6B	1.39	--	--
	Mean	1.40	1.70	0.93
18 weeks	7A	1.18	0.88	0.74
	8A	0.95	1.28	0.97
	17A	0.98	1.18	0.72
	Mean	1.04	1.11	0.81
24 weeks	6A	0.76	--	0.66
	9A	0.98	0.89	0.78
	14A	1.42	0.89	0.59
	16A	0.90	1.18	0.76
	22A	0.76	1.16	0.63
	Mean	0.96	0.95	0.68
51 weeks	1A	0.87	0.83	0.71

time the ratio between the pedicle and control bone reflects approximate differences in bone mineral per unit length of bone. Figure 9 is a plot of these ratios. MDP uptake peaks at about six weeks and thereafter declines toward control levels by 24 weeks. Blood flow increases more slowly than MDP, peaking at 9 - 10 weeks, then declining toward control levels by 24 weeks. The bone mineral shows a decrease by six weeks, then appears to recover at nine weeks, and then slowly declines to about 10% of control by 24 weeks when it appears to stabilize. It is interesting that MDP uptake shows the increase before blood flow has increased; otherwise blood flow and MDP uptake are temporally related as would be expected. Blood flow, however, shows a far greater increase than MDP uptake indicating that blood flow alteration is a sensitive indication of altered bone metabolism. Figures 10 - 19 show selected examples of the regional scans of MDP, microspheres and bone mineral. One can see that the region of greatest increase in blood flow is associated with the largest increase in MDP uptake; that is, below the epiphyseal cartilage plate. The region also demonstrates early bone mineral loss (six weeks) which is only seen in other areas with longer periods of immobilization. This region contains a large percentage of trabecular bone which is more metabolically active than cortical bone. Histologic and radiographic analysis also confirm this pattern of response. It is also interesting that the cut end of the pedicle bone does not show increased uptake of MDP. Radiographs show that this cut end gradually tapers to a point with the shaft becoming increasingly thin with time (Figure 20). This represents a reversal of the normal pattern with resorption occurring on the exterior and formation on the interior with resorption the predominant factor. This explains why MDP uptake is not elevated. It is evident that overall this model demonstrates significant increased resorption.

ORIGINAL PAGE
COLOR PHOTOGRAPH

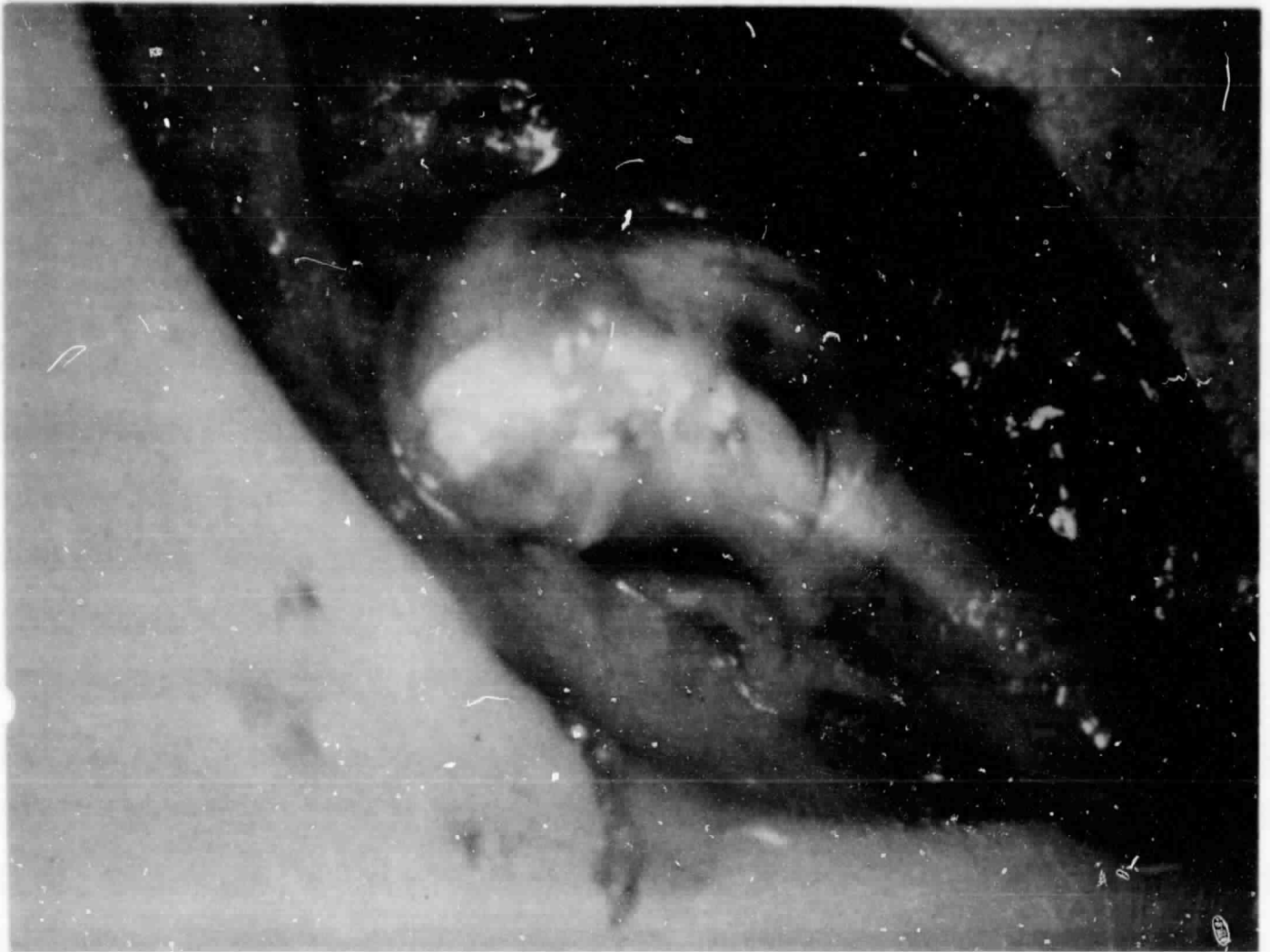
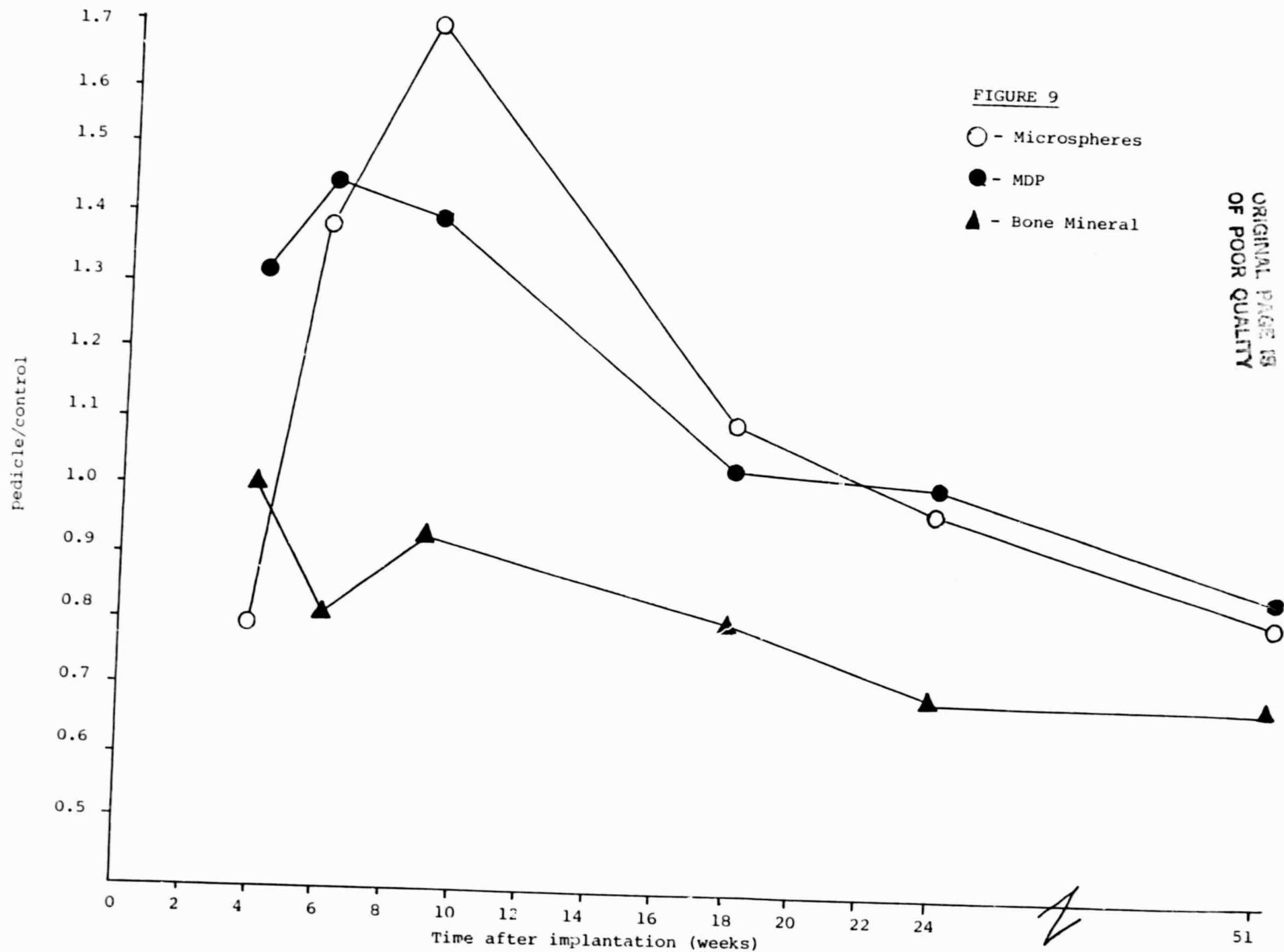


FIGURE 8 Pedicle graft 6 weeks after transplantation



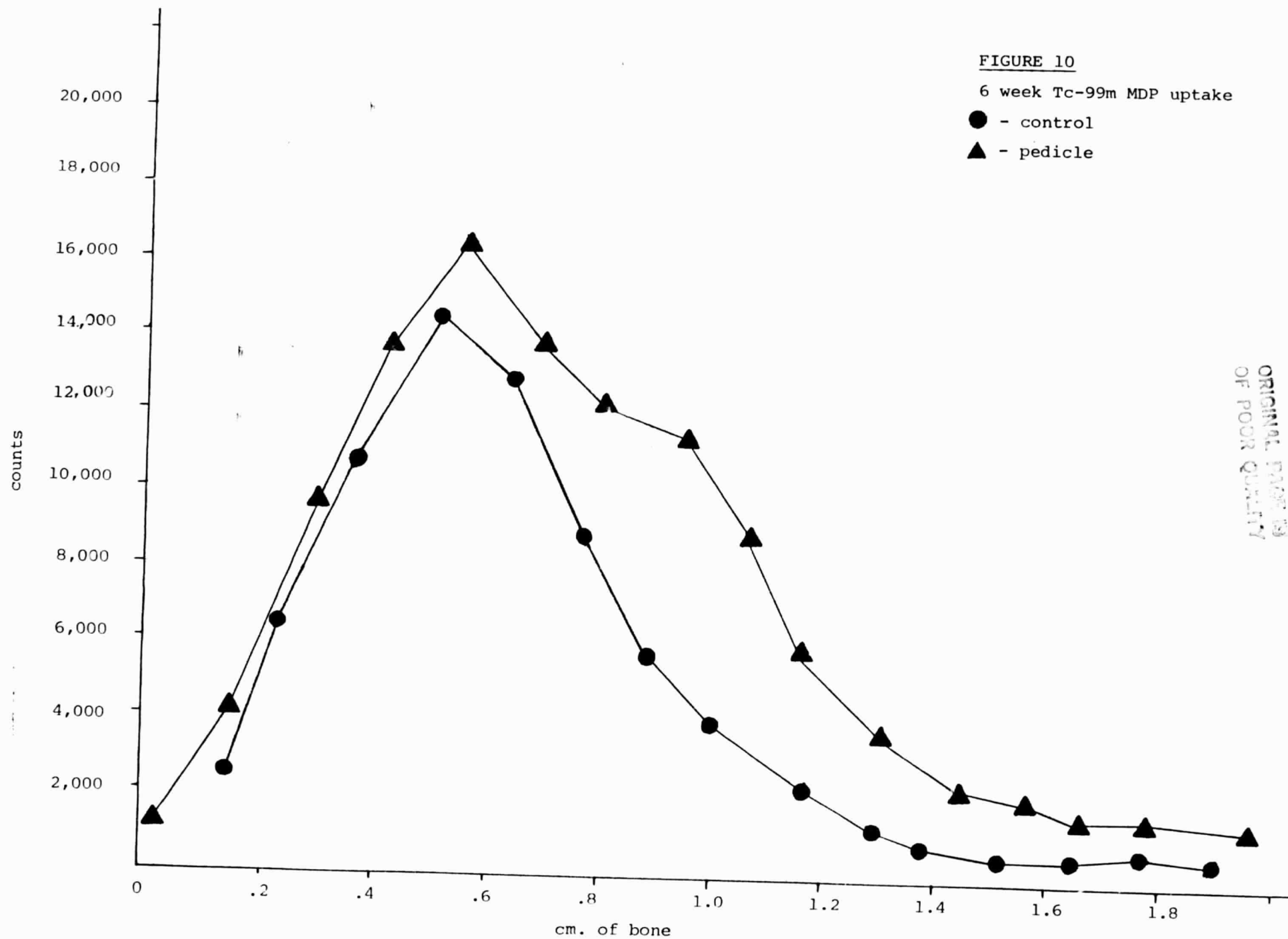
ORIGINAL PAGE IS
OF POOR QUALITY

FIGURE 10

6 week Tc-99m MDP uptake

● - control

▲ - pedicle



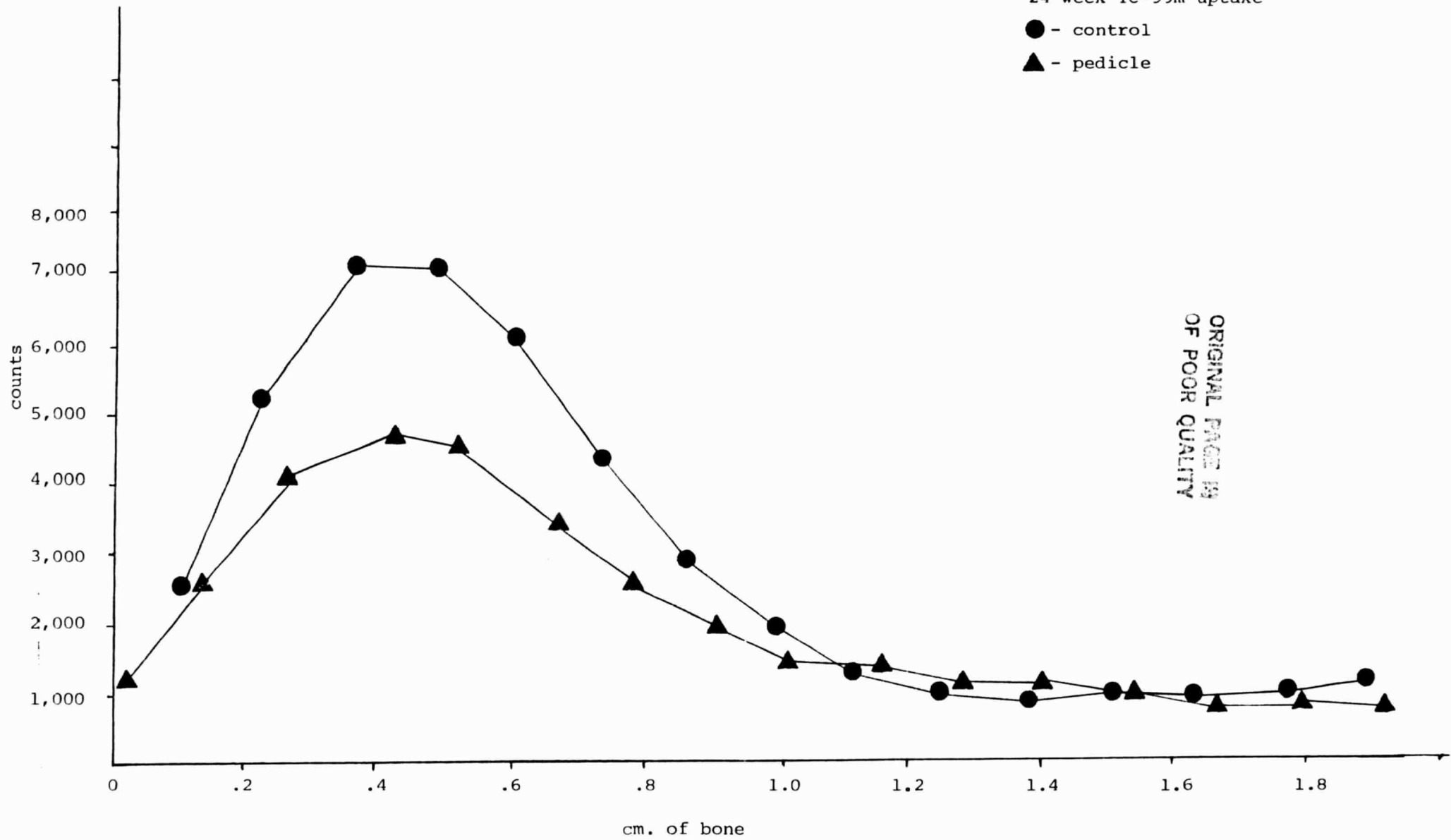
ORIGINAL PAGE IS
OF POOR QUALITY

FIGURE 11

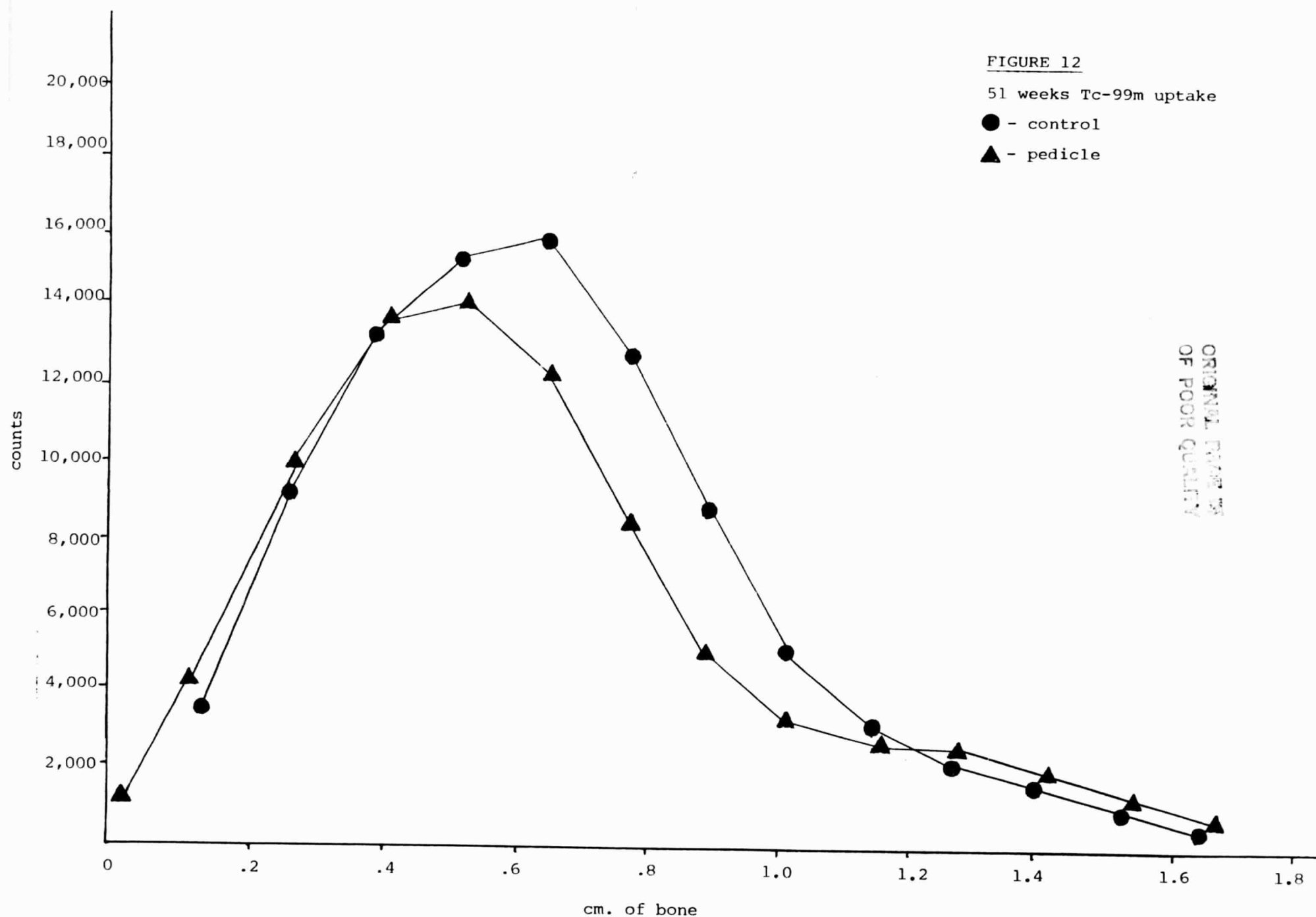
24 week Tc-99m uptake

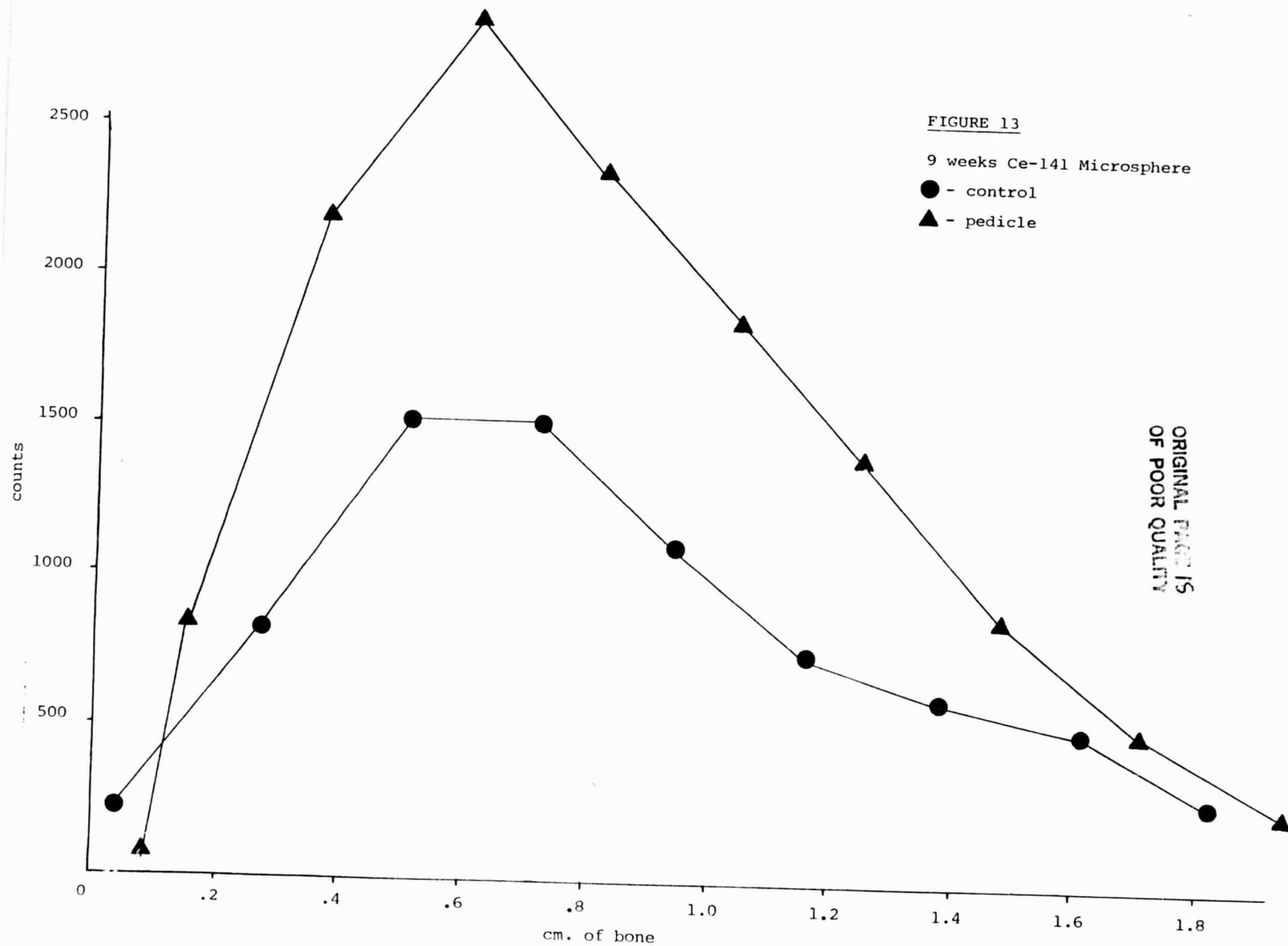
● - control

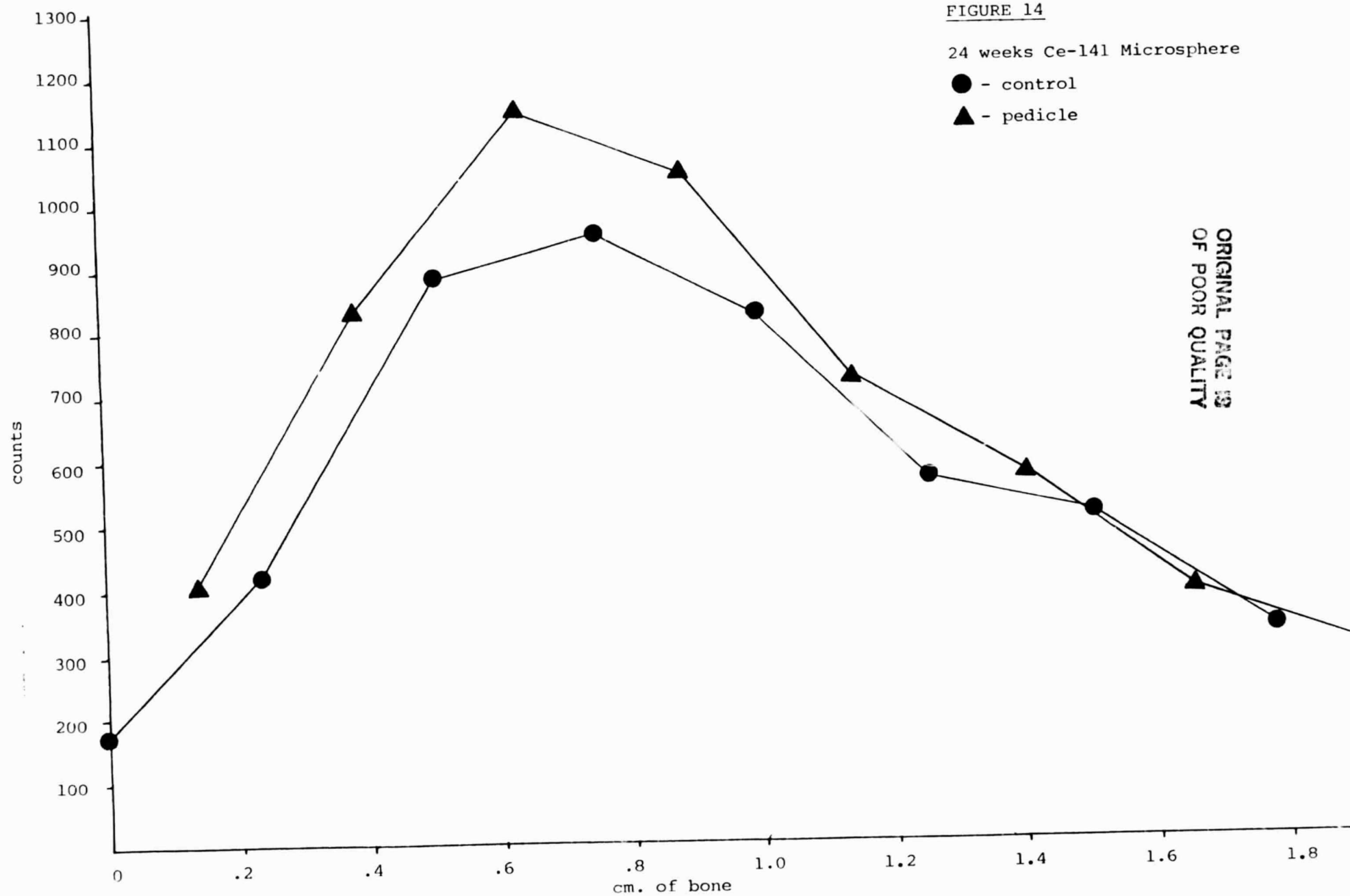
▲ - pedicle



ORIGINAL PAGE IS
OF POOR QUALITY



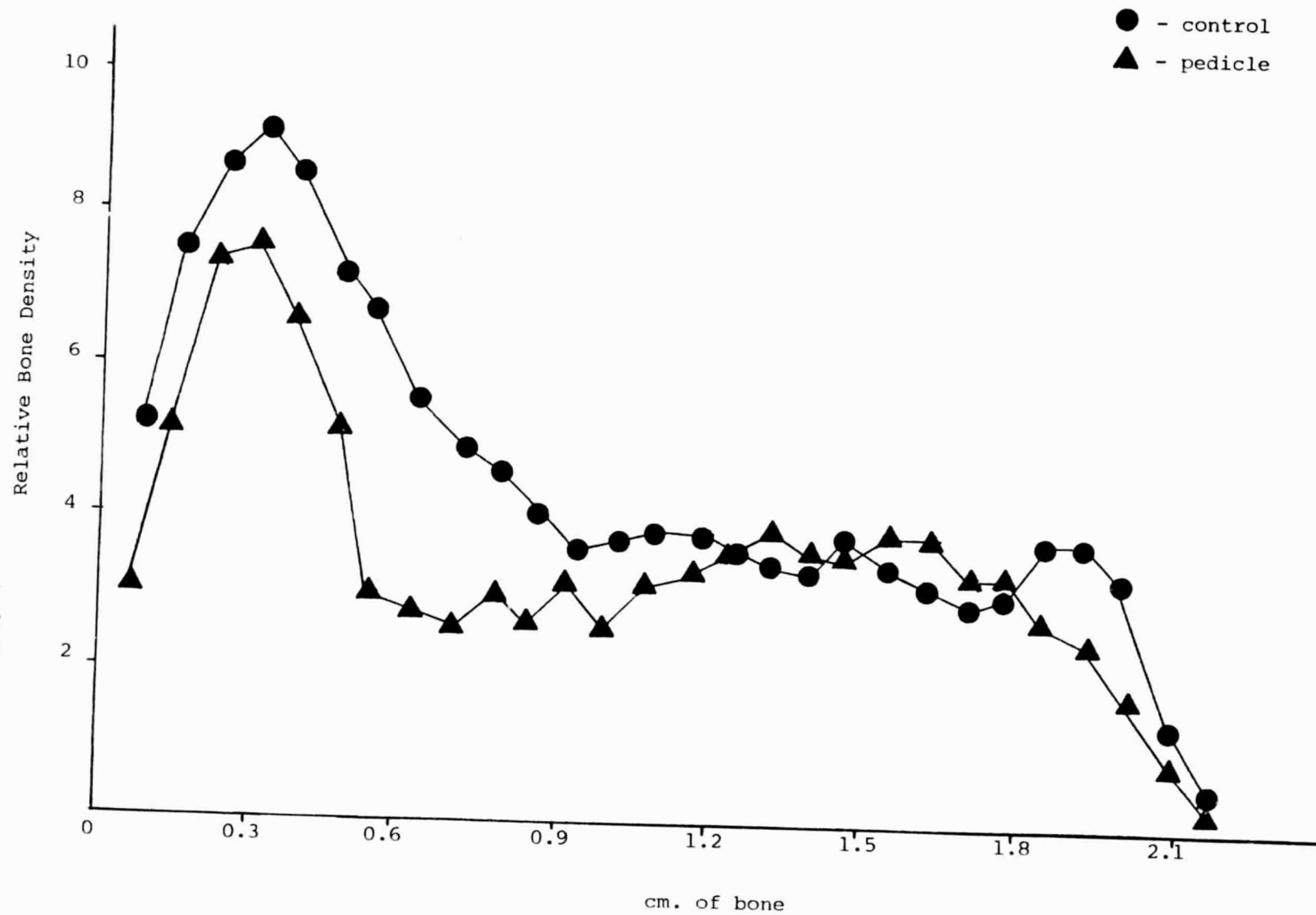




ORIGINAL PAGE IS
OF POOR QUALITY

FIGURE 15

6 week Bone Density Distribution



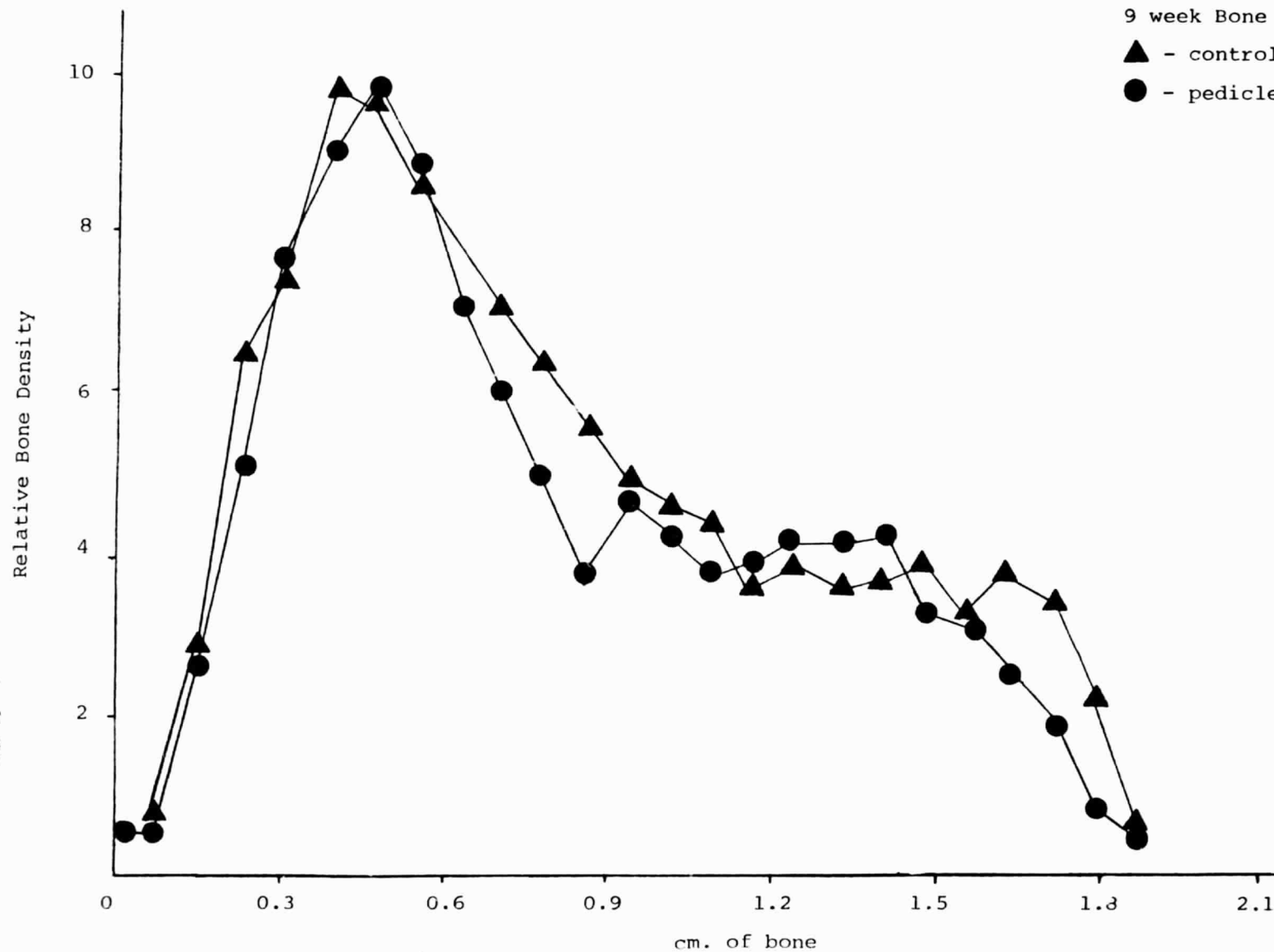
ORIGINAL PAGE IS
OF POOR QUALITY

FIGURE 16

9 week Bone Density Distribution

▲ - control

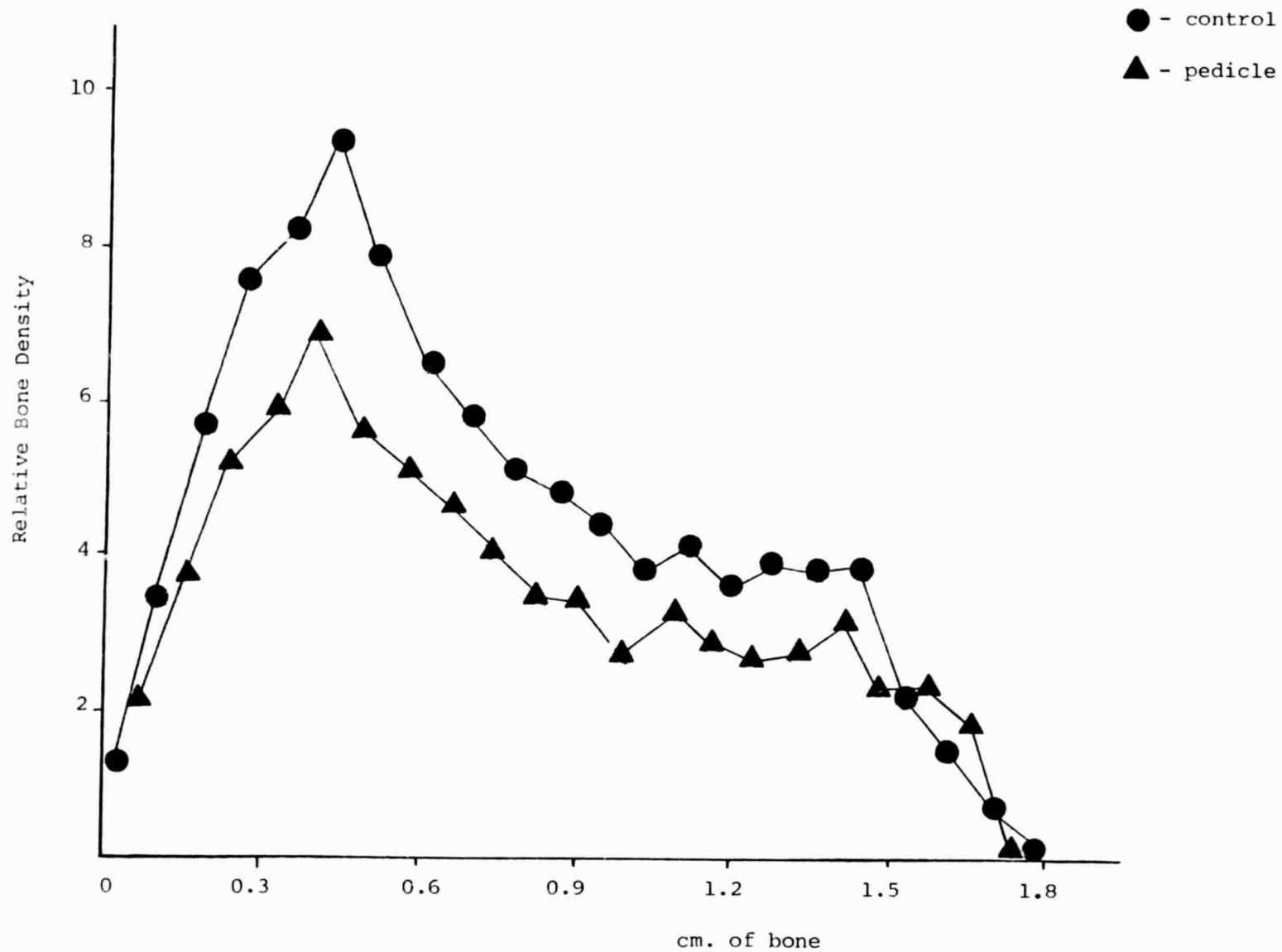
● - pedicle



ORIGINAL PAGE 19
OF POOR QUALITY

FIGURE 17

24 week Bone Density Distribution



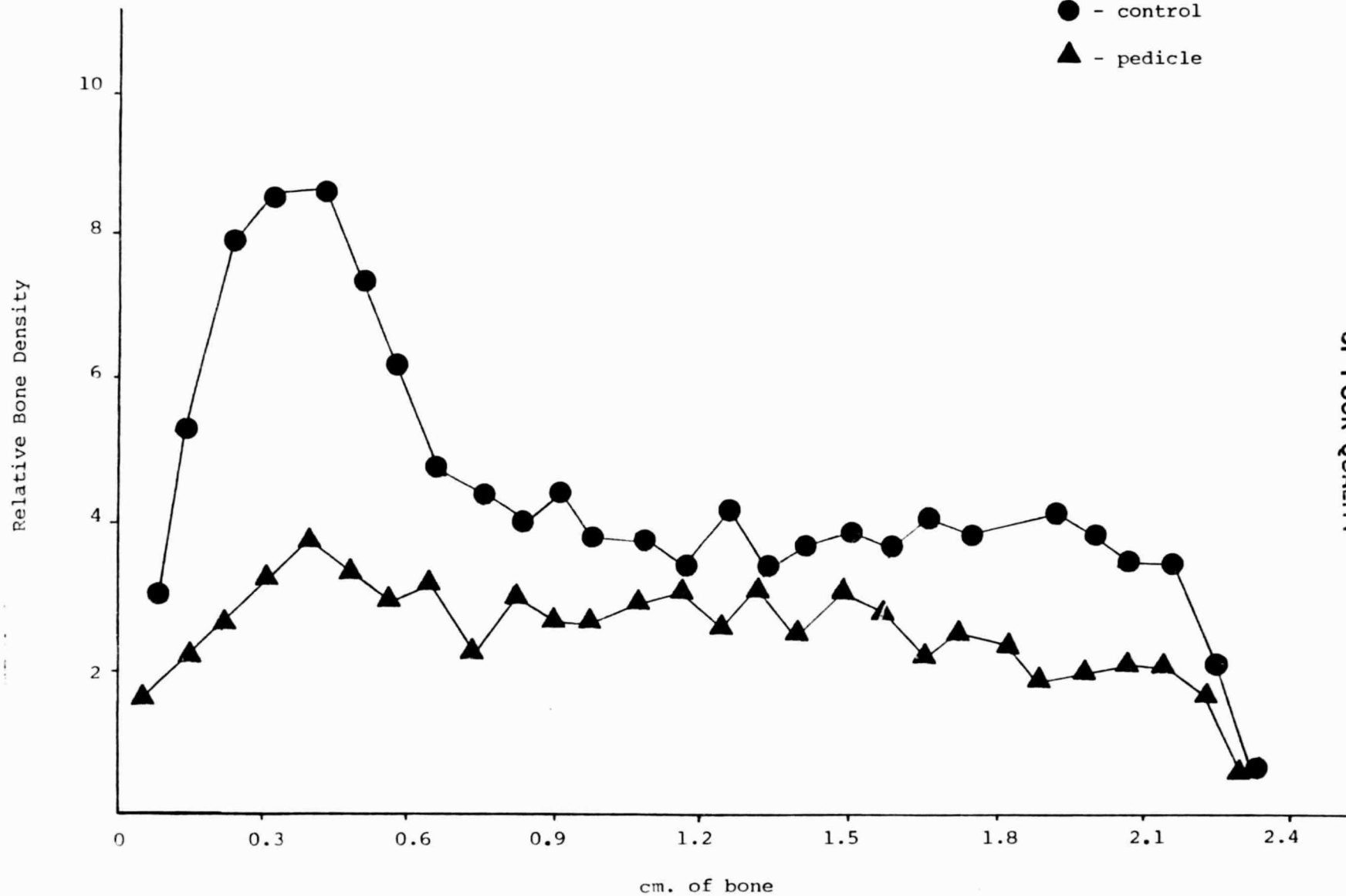
ORIGINAL PAGE IN
OF POOR QUALITY

FIGURE 18

24 week Bone Density Distribution

● - control

▲ - pedicle

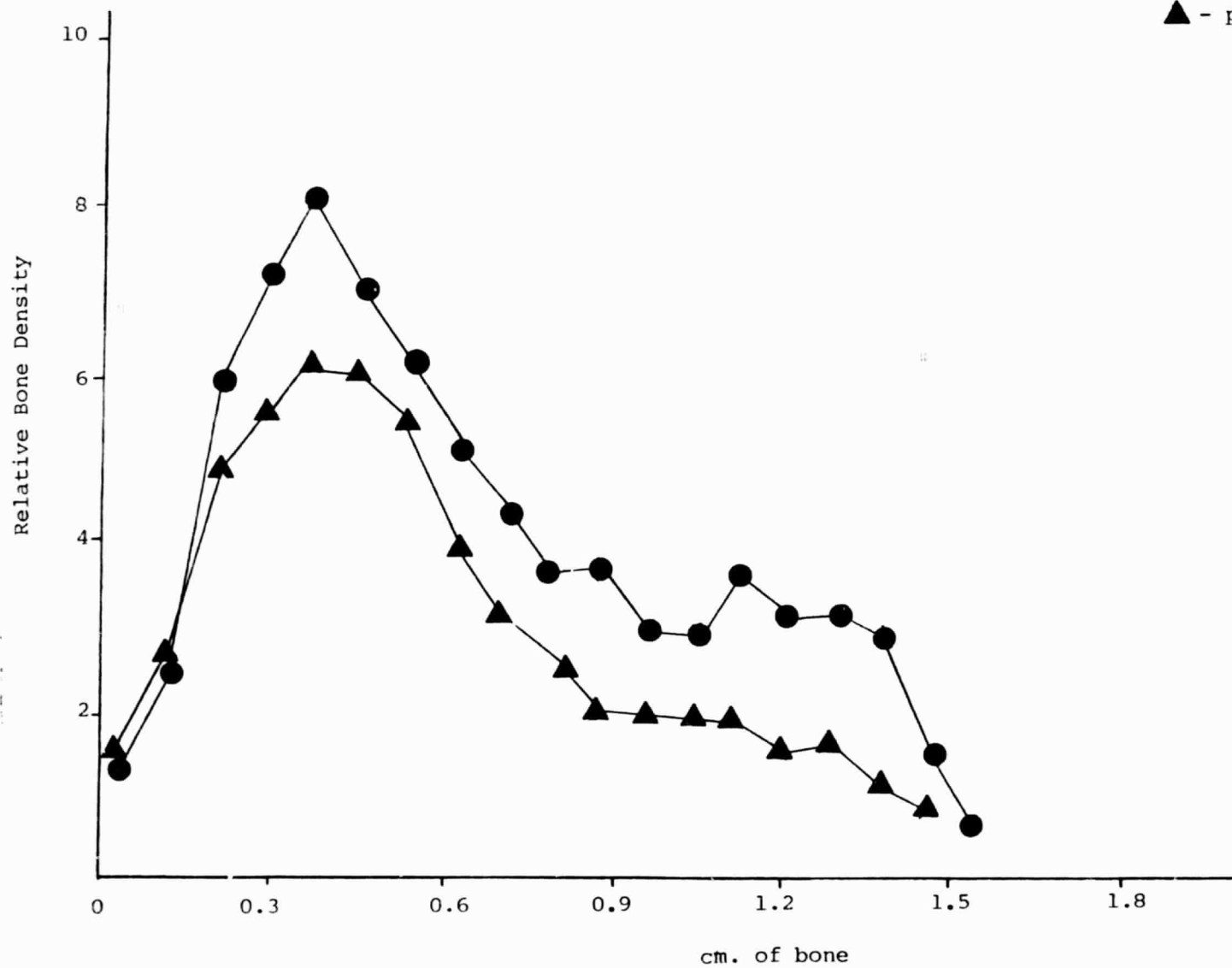


ORIGINAL PAGE IS
OF POOR QUALITY

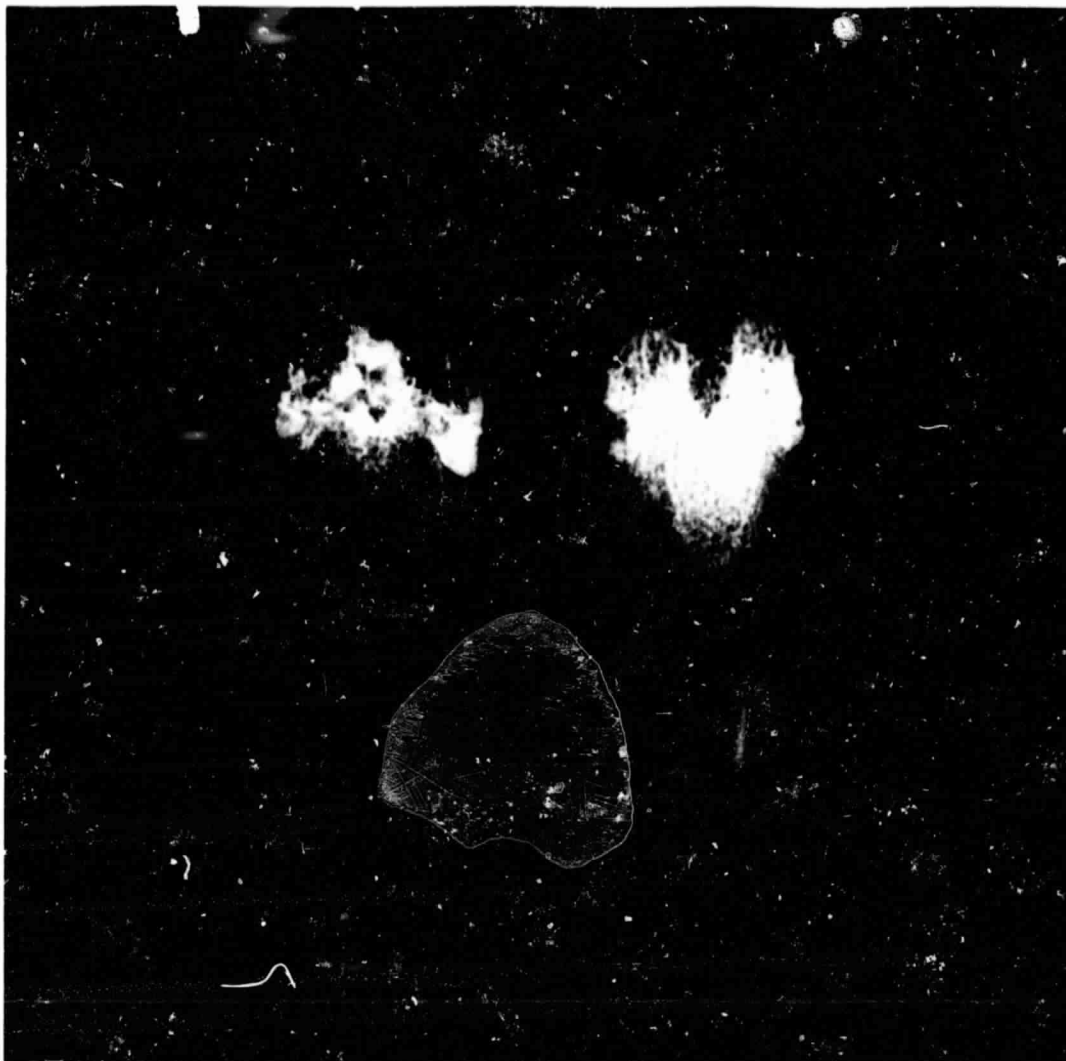
FIGURE 19

51 week Bone Density Distribution

● - control
▲ - pedicle



ORIGINAL PAGE IS
OF POOR QUALITY



ORIGINAL PAGE
COLOR PHOTOGRAPH

FIGURE 20 Transplanted pedicle and control bones
51 weeks after surgery.

PHASE II

Table 2 gives the bone mineral, MDP and microsphere uptake as a ratio of pedicle bone to control bone from the opposite limb. At six weeks both the clomiphene treated and the untreated animals show increased uptake of MDP and microsphere activity in the pedicle bone. This indicates that both metabolic activity and blood flow are elevated at this time and that clomiphene does not affect this response. A hard callous formed on the shaft of rat #2, accounting for the very high uptake value in this animal. This is commonly seen whenever the implant is allowed to touch viable bone. The BMC of the clomiphene treated animals shows no change at six weeks while the untreated animals lost about 13% total mineral. At 10 weeks MDP and microsphere ratios are now close to unity in both treated and untreated animals. The BMC in the treated animals indicates a loss of about 18% while the untreated animals average 29%. Clearly clomiphene retards resorption of bone. The regional BMC scans are shown in Figures 21 - 24. The six week scans demonstrate again the early trabecular bone loss seen in Phase I. The clomiphene treated animals do not show this loss. The 10 week control scans show a generalized loss compared to the six week animals. The bone loss distribution of 10 week clomiphene treated rats resembles that of the six week untreated rats.

Another series of animals have begun to corroborate these very important findings.

TABLE 2

RATIO OF PEDICLE TO CONTROL BONE

Six Weeks After Surgery

CLOMIPHENE TREATED				UNTREATED			
RAT#	MDP	SPHERES	BMC	RAT#	MDP	SPHERES	BMC
1	1.6	1.7	0.99	4	1.6	---	0.81
2	3.9*	---	1.06	5	1.7	1.4	0.88
3	1.6	---	1.02	6	1.3	1.5	0.92
\bar{x}	2.4	1.7	1.02	\bar{x}	1.5	1.4	0.87

Ten Weeks After Surgery

CLOMIPHENE TREATED				UNTREATED			
RAT#	MDP	SPHERES	BMC	RAT#	MDP	SPHERES	BMC
7	0.9	---	0.82	10	0.9	1.2	0.72
8	1.2	1.1	0.88	11	1.1	0.9	0.70
9	1.1	1.2	0.83				
\bar{x}	1.1	1.2	0.84	\bar{x}	1.0	1.0	0.71

*Extra calcification on shaft

ORIGINAL PAGE IS
OF POOR QUALITY

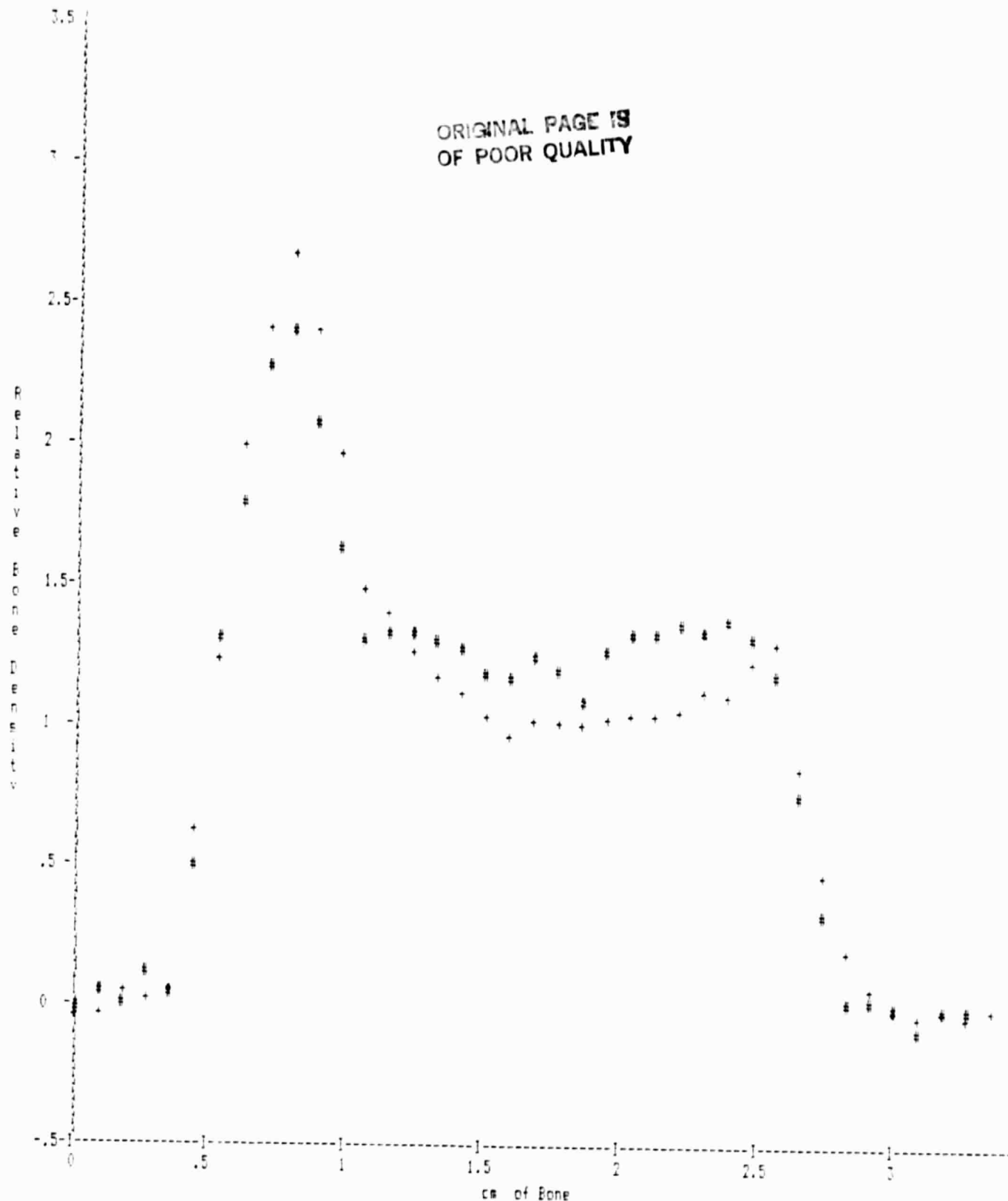


FIGURE 21 Six week pedicle grafts, clomiphene treated
- pedicle bone
+ - femur bone from opposite limb

ORIGINAL PAGE IS
OF POOR QUALITY

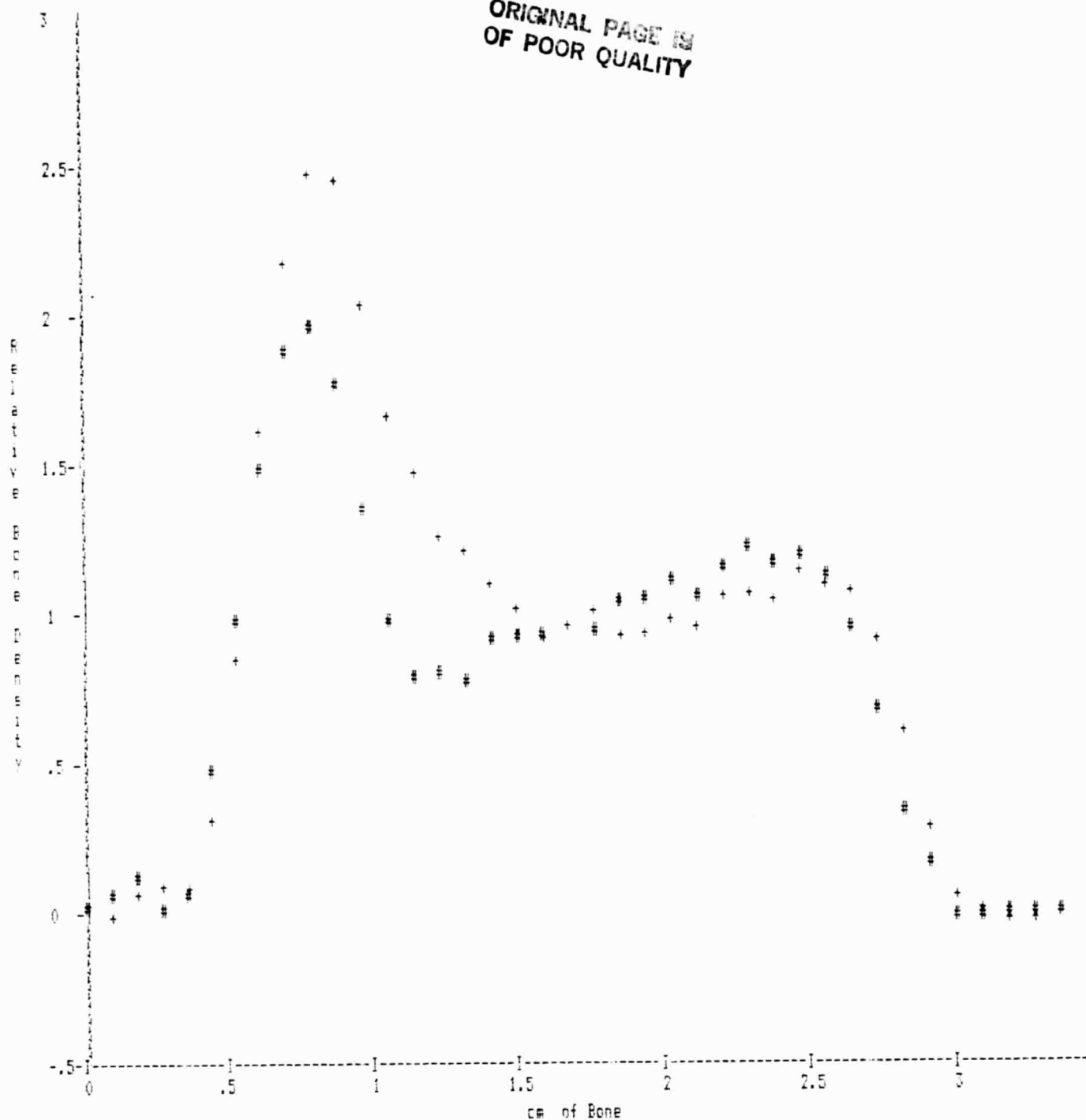


FIGURE 22 Six week pedicle grafts, untreated
- pedicle bone
+ - femur bone from opposite limb

ORIGINAL PAGE IS
OF POOR QUALITY

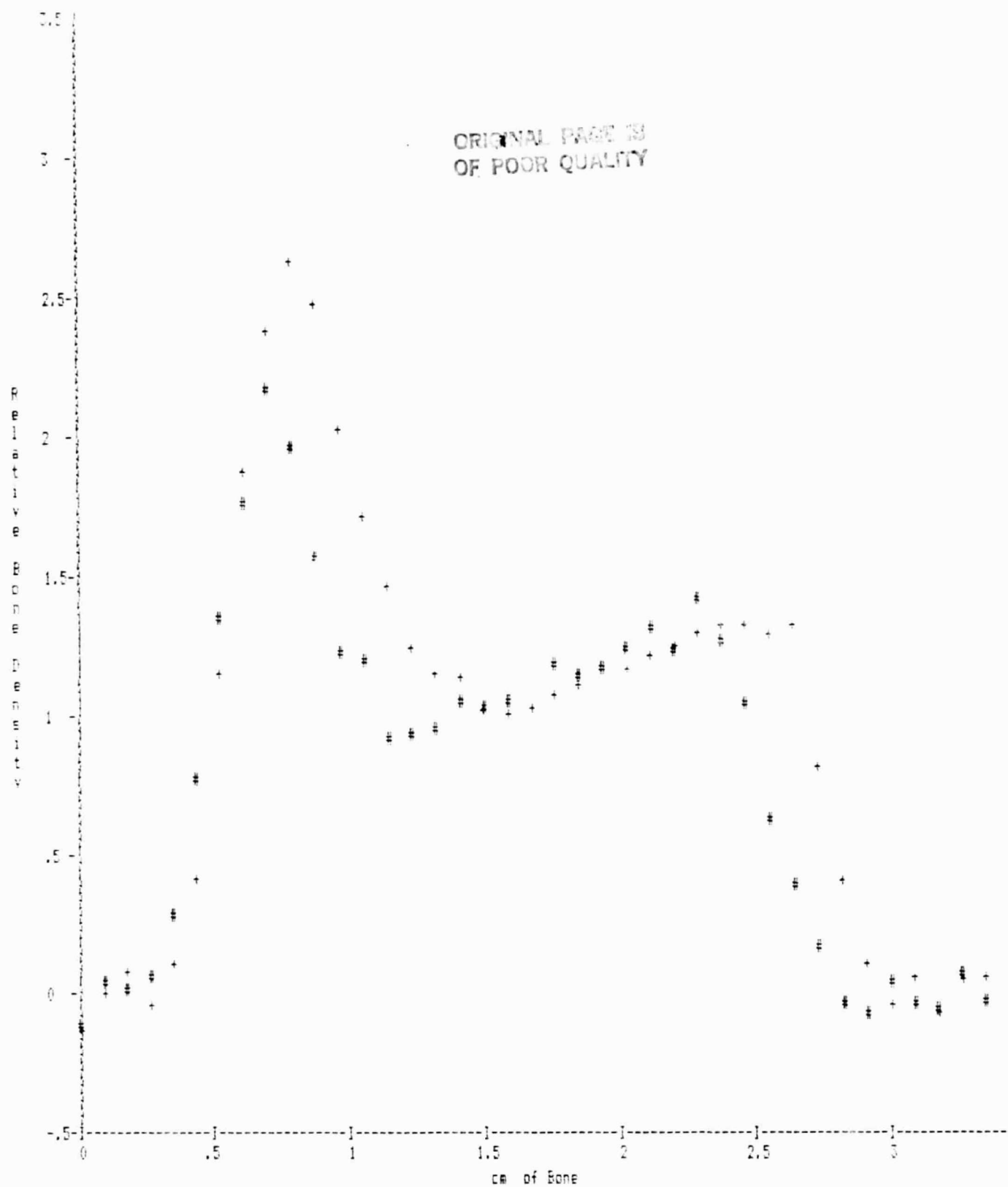


FIGURE 23 Ten week pedicle grafts, clomiphene treated
- pedicle bone
+ - femur bone from opposite limb

ORIGINAL PAGE IS
OF POOR QUALITY

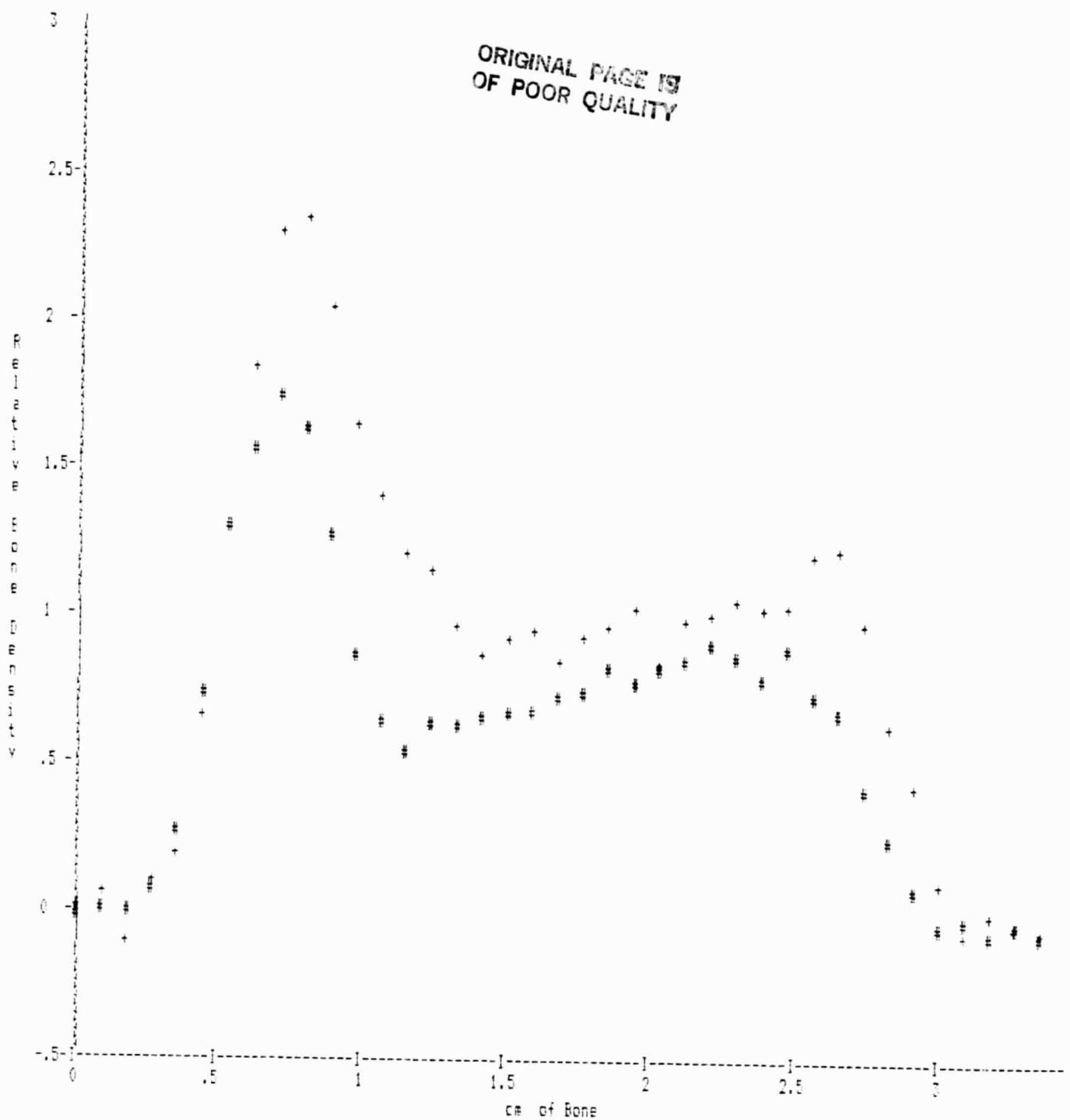


FIGURE 24 Ten week pedicle grafts, untreated
- pedicle bone
+ - femur bone from opposite limb

SUMMARY

An animal model was developed for transplantation of bone on a nutrient vascular pedicle. This model, which uses Sprague-Dawley rats, entails resecting the distal half of the femur with placement (i.e. "transplantation") of the resected femur, on its nutrient vascular pedicle, into the groin of the animal. We have shown that this model undergoes no necrosis or creeping substitution, making it ideal for this study. The very uniqueness of the experimental model makes it ideally suited for a study of bone metabolism under conditions which closely simulate those which may be encountered in long term space flight.

This unique model has a number of advantages for studying a wide variety of factors which could alter bone metabolism. Most animal models used to examine bone disuse necessitate some form of restraint, e.g. casting or suspension, which invariably produces stress with accompanying physiological changes. Using this particular transplanted bone model, stress producing immobilization of the animal is not required. The technique however, requires a skilled surgeon to perform the required surgery, without which the failure rate is high.

The first phase of the study was to determine the long term course of these implants measuring regional and total bone mineral, blood flow, and Tc-99m labeled MDP uptake. The magnitude and time course of these changes was presented.

The second phase tested the hypothesis that clomid, an estrogen agonist/antagonist would prevent the rapid trabecular and cortical resorption of pedicle bone normally seen. This study presents conclusive evidence that this drug will protect bone from disuse loss of mineral by retarding this resorption.

REFERENCES

1. Smith, M.C., Rambaut, P.C., Vogel, J.M., Whittle, M.W.: Bone mineral measurement, Experiment M078. In: Biomedical Results of Skylab. Richard S. Johnston and Lawrence F. Dietlein, Editors. NASA, Washington, D.C., 1977.
2. Tilton, F.E., Degioanni, J.C., Schneider, V.S.: Long-term follow-up of Skylab bone demineralization. J. Aviat. Sp. and Envir. Med. unpublished.
3. Wolff, J.: Das gesetz der tranformation der knochen. Berlin A. Hirshchwald, 1892.
4. Schneider, V.S.: Personal Communication.
5. Lockwood, D.R., Vogel, J.M., Schneider, V.S., Hulley, S.B.: Effect of diphosphonate EHDP on bone mineral metabolism during prolonged bed rest. J. Clin. Endo. Met. 41: 533, 1975.
6. Vogel, J.M., Whittle, M.W.: Bone mineral changes: The second manned Sklab mission. Aviat. Sp. Environ. Med. 47: 396, 1976.
7. Thornton, W.B., Rummel, J.A.: Muscular deconditioning and its prevention in space flight. In: Biomedical Results of Skylab. Richard S. Johnston and Lawrence F. Dietlein, Editors. NASA, Washington, D.C., 1977.
8. Whedon, G.D., Lutwak, L., Reid, J., Rambaut, P.C., Whittle, M., Smith, M.C., Leach, C.S.: Mineral and nitrogen metabolic studies on Skylab orbital space flights. Trans. Assn. Amer. Phys. 137: 95, 1974.
9. Smith, M.D., Rambaut, P.C.: Skylab nutrition studies. In: Cospar Life Sciences and Space Research, R. Homquist and A.C. Strickland, Editors, Pergamon Press, Oxford and New York, 1977, P. 193.
10. Hegsted, M., Schuette, S., Zemel, M., and Linkswiler, H.M.: The effect of level of protein and phosphorus intake on calcium balance in young adult men. Fe. Proc. 38: 765, 1979.
11. Leach, C.S., Rambaut, P.C.: Biochemical observations of long duration manned orbital space flight. J. Amer. Med. Wom. Assn. 30: 153, 1975.
12. Weksler, W.R., Henry, H.L., and Norman, A.W.: Studies on the mode of action of calciferol. Arch. Biochem. Biophys. 183: 168, 1977.
13. Morey, E.R., Baylink, D.J.: Inhibition of bone formation during space flight. Science (in press).
14. Yagodovsky, V.S., Trifranidi, L.A., and Gorklova, G.P.: Space flight effects on skeletal bones of rats. Aviat. Sp. Environ. Med. 47: 734, 1976.
15. Morey, E.R.: Personal Communication.
16. Schraer, H., Siar, W.J., and Schraer, R.: Changes in bone mass and density in living rats during the manipulation of calcium intake. Arch. Biochem. Biophys. 100: 393-398, 1963.

17. Handler, P., Baylin, G.J., Follis, R.H.: The effects of caloric restriction on skeletal growth. *J. Nutrition*, 34: 677, 1947.
18. Marino, A., Becker, R.O., Hart, F.X., and Anders, F.: Space osteoporosis: an electromagnetic hypothesis.
19. Wever, R.: ELF effects on human circadian rhythms. In: *ELF and VLF electromagnetic field effects*. M.A. Persinger, Editor. Plenum Press, New York, P. 101, 1974.
20. Hamer, J.R.: Effects of low level, low frequency electric fields on human reaction time. *Commun. Behav. Biol.*, Part A 2:217-222, 1968.
21. Novall, J.J., Sohler, A., Reisberg, R.B., Coyne, H., Straub, K.D., McKinney, H.: Extremely low frequency electric field induced changes in rate of growth and brain and liver enzymes of rats. *Compilation of Navy sponsored ELF Biomedical and Ecological Research Reports*, Vol. III. Naval Medical Research and Development Command, Bethesda, MD. NTIS No. AD A035959, 1976.
22. Marino, A.A., Cullen, J.M., and Becker, R.O.: Power frequency electric fields and biological stress: A cause and effect relationship. In: *Proceedings of the 18th Annual Hanford Life Sciences Symposium: Biological Effects of Extremely Low Frequency Electromagnetic Fields*. Battelle Memorial Institute/Pacific Northwest Laboratories, Richland, WA., 1978.
23. Ostrup, L.: Rib transplantation. In: *Microsurgical Composite Tissue Transplantation*, p.424. Edited by Donald J. Serafin, C.V. Mosby, St. Louis, 1979.
24. Ostrup, L., Fredrickson, J.M.: Distant transfer of a free, living bone graft by microvascular anastomoses: An experimental study. *Plast. Reconstr. Surg.*, 54: 247, 1974.
25. Katz, D.: Bone growth in young rat epiphysis. In publication.
26. Katz, D., Teot, L., Spira, M.: Epiphyseal Growth of Vascularized bone grafts in young rats. In: *Proceedings of the American Society of Plastic and Reconstructive Surgeons*, New York, Sept. 1981.
27. Cameron, J.R., Sorenson, J.A.: Measurement of bone mineral in vivo: An improved method. *Science*, 142: 230-232, 1963.
28. Trueta, J., Morgan, J.D.: The vascular contribution of osteogenesis: Studies by the injection method. *Brit. J. Bone and Jt. Surg.* 42: 97, 1960.
29. Bell, W.H.: Revascularization and bone healing after anterior maxillary osteotomy: A study using adult Rhesus monkeys. *J. Oral Surg.* 42: 97, 1960.
30. Clark, J., Markaverich, B.: The agonistic-antagonistic properties of clomiphene: A review. *Pharmac. Ther.* In press, 1982.